S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE
(DEF)

RISK CHARACTERIZATION DOCUMENT

Medical Toxicology and Worker Health and Safety Branches

Department of Pesticide Regulation

California Environmental Protection Agency

November 3, 1998

S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE

EXECUTIVE SUMMARY

Introduction

S,S,S-Tributyl phosphorotrithioate (DEF) is an organophosphate chemical which was first registered in 1960 for cotton defoliation (U.S. EPA, 1981). In 1981, the U.S. EPA issued a decision not to initiate a Rebuttable Presumption Against Registration (RPAR) review of DEF despite evidence of irreversible neurotoxic effects in laboratory animals exposed to DEF. This decision was based on the lack of evidence of neurotoxic symptoms among applicators exposed to DEF and adequate margins of exposure assuming protective clothing is worn. An evaluation of the potential dietary exposure to DEF residues was conducted by the Department of Pesticide Regulation (DPR) in the California Environmental Protection Agency under the provisions of AB2161 (Bronzan). This Risk Characterization Document addresses the potential adverse human health effects associated with both occupational and dietary exposure to DEF.

The Risk Assessment Process

The risk assessment process consists of four aspects: hazard identification, dose-response assessment, exposure assessment, and risk characterization.

Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and estimates the amount which could potentially cause an adverse effect. The amount which will not result in an observable or measurable adverse effect is the No-Observed-Effect Level, NOEL. A basic premise of toxicology is that at a high enough dose, virtually all substances will result in some toxic manifestation. Chemicals are often referred to as "dangerous" or "safe," as though these concepts were absolutes. In reality, these terms describe chemicals which require low or high dosages, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the types of toxic effects which can be caused, and the exposure levels (doses) at which effects may be seen. State and federal testing requirements mandate that substances be tested in laboratory animals at doses high enough to produce toxic effects, even if such testing involves chemical levels many times higher than those to which people might be exposed.

The exposure assessment includes an estimation of the potential occupational and dietary exposure through the oral, dermal and inhalation routes on an acute (one time), subchronic (seasonal), and chronic (long-term) basis. Occupational exposure is based on the amount of pesticide residue in the air, on clothing, and on the skin. The exposure is adjusted for the number of hours worked per day, body weight, dermal absorption rate and breathing rate. For dietary exposure, the levels of exposure are determined by the amount of pesticide residue on specific commodities and processed foods, and the consumption rate.

The risk characterization then integrates the toxic effects observed in the laboratory studies, conducted with high dosages of pesticide, to potential human exposures to low dosages of pesticide residues through agricultural work or in the diet. The potential for possible non-carcinogenic adverse health effects in human populations is expressed as the margin of

exposure (MOE), which is the ratio of the dosage which produced no effect in laboratory studies to the estimated occupational or dietary dosage. For carcinogenic effects, the probability of risk is calculated as the product of the carcinogenic potency of the pesticide and the estimated lifetime occupational or dietary exposure.

Toxicology

DEF appears to be readily absorbed by the oral route and rapidly metabolized in the species examined. Several metabolic pathways have been proposed for DEF based on a few metabolites; however, the metabolism of DEF by the various routes of exposure is still highly speculative. One explanation for the inability to identify metabolites was that most of the parent compound had been extensively metabolized into natural constituents, such as fatty acids and proteins. n-Butyl mercaptan (nBM) was identified in the excreta of hens administered DEF orally. It was proposed that DEF was hydrolyzed to nBM in the gut. nBM is thought to be a product of the normal metabolism of DEF in tissues. DEF also readily degrades to nBM in the environment and may be responsible for complaints by residents in communities near cotton fields due to its strong skunk-like odor (odor threshold ~ 0.01 to 1 ppb). However, limited data on nBM preclude a thorough toxicological evaluation.

The acute effects of DEF in experimental animals are due primarily to its inhibition of various enzymes in the nervous system including cholinesterase (ChE) and neuropathy target esterase. The clinical signs observed include both cholinergic signs (lacrimation, salivation, diarrhea, pupil constriction, hypothermia, twitching, tremors, convulsions) and delayed neuropathy (loss of coordination and paralysis), although delayed neuropathy was observed primarily in hens exposed by the dermal route. Anemia was also observed after acute exposure to DEF. The anemia was probably due to the nBM metabolite of DEF which interferes with the normal metabolism in red blood cells and ultimately leads to the destruction of the cell. The anemia was observed at higher dosages than the neurological effects with acute and subchronic exposure. Although the technical grade DEF was only mildly irritating to the eyes and skin, the DEF formulation is corrosive to the skin and is a severe irritant to eyes.

The neurological effects were also the predominant effects seen in animals with daily exposure from 3 weeks to 3 months, although anemia, impaired retinal function and microscopic changes in the adrenal gland were also observed at the same dose level that neurological effects occurred in one rat study. In a rat reproductive toxicity study, several effects were seen, including reduced fertility, increased gestation length, increased number of stillbirths, reduced birth weights, increased postnatal deaths, and discolored livers in offspring. However, the neurological effects were the most sensitive endpoints.

Adverse effects observed in animals with exposure to DEF for 1 to 2 years included reduced body weights, hypothermia, brain ChE inhibition, and anemia. Anemia was seen in mice, rats, and dogs. Brain ChE inhibition was detected in rats and mice. The reduced weight gain and hypothermia were only observed in rats. In mice, numerous non-carcinogenic microscopic changes in the gastrointestinal tract, liver, adrenal gland, and spleen were seen. Several potentially precancerous microscopic changes were also observed in the small intestine and lung. Microscopic changes in the small intestine, liver, and adrenal glands were also observed in rats. In addition, numerous ocular effects were observed in one rat study, the most significant being bilateral retinal atrophy, and optic nerve atrophy. The corneal opacity, cataracts, and optic nerve atrophy appear to be secondary to the degenerative changes in the retina. The most sensitive endpoints were the anemia and microscopic changes in the small intestine.

There was a dose-related increase in malignant tumors (adenocarcinomas) of the small intestine of both sexes, malignant tumors (hemangiosarcomas) in the liver in males, and benign lung tumors (alveolar/bronchiolar adenomas) in females in a mouse study which suggests that DEF may be carcinogenic to humans. There were no dose-related increases in tumors in two rat chronic studies and all the genetic toxicity studies for DEF were negative. Because the increase in tumors occurred in both sexes of mice at multiple sites (one of which was rare), a quantitative assessment of the carcinogenic potency was conducted based on the incidence of hemangiosarcomas in male mice.

Exposure Analysis

Daily, seasonal, and lifetime exposure dosages were estimated for 9 different job categories of pesticide workers potentially exposed to DEF. Five of these job categories are handlers (aerial mixer/loaders, pilots, flaggers, ground mixer/loaders and ground applicators) and four are harvesters (picker operator, module builder operator, raker, and tramper). Seasonal exposure was estimated assuming the pesticide workers were exposed for an average of 21 days during a 45-day use season. The lifetime exposure was estimated, assuming a worker is exposed for 40 years of a 70-year lifespan. Combined occupational, dietary and ambient air exposure was initially evaluated. However, since the dietary and ambient air exposure were minor compared to occupational exposure, no further analysis was performed.

Dietary exposure to DEF may occur from consumption of cottonseed products, such as cottonseed oil or cottonseed meal, or from consumption of meat or milk from livestock that were fed cottonseed products or cotton gin trash in their feed. Most of the residue data for DEF is for whole cottonseed. The limited residue data in processed cottonseed products, meat and milk was used to derive processing and distribution factors which were then applied to the residues in whole cottonseed. The potential dietary exposure was estimated for various population subgroups using anticipated residues derived from actual residues on whole cottonseed. Children, 1 to 6 years old, had the highest potential acute and chronic dietary exposure to DEF. Combined dietary and ambient air exposure was evaluated for the general population. Children, 1 to 6 years old, also had the highest combined exposure.

Risk Characterization

The risk for non-carcinogenic health effects in humans is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from animal studies to the human exposure dosage. The MOEs ranged from approximately 40 to 1,100 for acute systemic effects and 1,500 to 37,000 for acute dermal irritation. The seasonal MOEs ranged from 17 to 86 for most pesticide workers, except ground applicators whose MOE was greater than 400. The estimated carcinogenic risk from occupational exposure to DEF ranged from 4 to 30 excess cancer cases in 100,000 people for most pesticide workers, except for ground applicators whose estimated risk was between 7 and 12 excess cancer cases in a million people.

The MOEs for acute dietary exposure to DEF in the various population subgroups ranged from 5,000 to 20,000 using anticipated residues in cottonseed products. The MOEs for chronic dietary exposure ranged from 1,400 to 10,000. The MOEs for combined acute dietary and ambient air exposure to DEF ranged from 3,800 to 12,000. The MOEs for combined chronic dietary exposure ranged from 1,100 to 4,200. The estimated carcinogenic risk from dietary exposure to DEF was between 2 and 4 excess cancer cases in a million people. The

estimated carcinogenic risk from combined dietary and ambient air exposure was between 3 and 5 excess cancer cases in a million people.

Tolerance Assessment

Acute dietary exposure to DEF was also evaluated assuming commodities were consumed at the tolerance levels. The MOEs for various population subgroups ranged from 3,300 to 11,000. Chronic consumption of commodities containing DEF residues at the tolerance level was considered highly improbable based on the small percentage of samples (<1%) that had residues at or above tolerance in the DPR pesticide monitoring programs. Therefore, a tolerance assessment for chronic dietary exposure to DEF was not conducted.

Conclusions

Generally, a margin of exposure greater than 100 is considered protective of human health when it is calculated from a NOEL derived from an animal study. The MOEs for acute dermal irritation were greater than 1,000 for all pesticide workers. The MOEs for acute systemic effects were greater than 100 for workers involved in application of DEF, except for mixer/loaders in ground application. The acute MOEs for cotton harvesters were less than 100, except for module builder operators. The seasonal MOEs were less than 100 for most pesticide workers, except ground applicators. The estimated carcinogenic risks from occupational exposure were approximately one excess cancer case per 10,000 people for most pesticide workers, except ground applicators whose estimated risk was approximately one excess cancer case in 100,000 people.

The MOEs for acute and chronic dietary exposure to DEF in various population subgroups were greater than 100 using either anticipated residue levels or the tolerance levels. The acute and chronic MOEs for combined dietary and ambient air exposure were also greater than 100. The estimated carcinogenic risk from dietary exposure ranged from 2 to 4 excess cancer cases in a million people. However, the chronic dietary exposure is based entirely on anticipated residues that were estimated from whole cottonseed using processing and distribution factors. The chronic dietary exposure may have been overestimated due to several assumptions including not adjusting the residue levels in cottonseed oil for the deodorization process, assuming cattle consumed cottonseed by-products in their feed at the maximum allowable level on a long-term basis, and not correcting for the percent of crop treated.

Contributors and Acknowledgments

Principal Author:	Carolyn M. Lewis, MS, DABT Associate Toxicologist Health Assessment Section
Toxicology Reviews:	Gary Patterson, PhD Supervising Toxicologist Medical Toxicology Branch
	Thomas P. Kellner, PhD, DABT Staff Toxicologist Product Data Review Section
Worker Exposure Assessment:	Tareq A. Formoli, MS Associate Environmental Research Scientist Worker Health and Safety Branch
	Rhoda G. Wang, PhD Staff Toxicologist Worker Health and Safety Branch
Peer Reviewed By:	Earl F. Meierhenry, DVM, PhD, ACVP Staff Toxicologist Health Assessment Section
	Nu-may R. Reed, PhD, DABT Staff Toxicologist Health Assessment Section
	Keith Pfeifer, PhD, DABT Senior Toxicologist Health Assessment Section
	Jay P. Schreider, PhD Primary State Toxicologist Medical Toxicology Branch

DPR acknowledges the review of this document by the Pesticide and Environmental Toxicology Section, Office of Environmental Health Hazard Assessment, as part of the Adverse Effects Advisory Panel evaluation.

TABLE OF CONTENTS

I. SUMMARY

S,S,S-Tributyl phosphorotrithioate (DEF) is an organophosphate chemical which was first registered in 1960 for cotton defoliation (U.S. EPA, 1981). DEF induces early leaf abscission through changes in the levels of plant hormones. Defoliation occurs 4 to 7 days after treatment. Dietary exposure to DEF may occur from consumption of cottonseed products such as cottonseed oil or cottonseed meal or from consumption of meat or milk from livestock that are fed cottonseed products in their feed. In 1981, the U.S. EPA issued a decision not to initiate a Rebuttable Presumption Against Registration (RPAR) review of DEF despite evidence of irreversible neurotoxic effects in laboratory animals exposed to DEF. This decision was based on the lack of evidence of neurotoxic symptoms among applicators exposed to DEF and adequate margins of exposure assuming protective clothing is worn. An evaluation of the potential dietary exposure to DEF residues was conducted by the Department of Pesticide Regulation (DPR) in the California Environmental Protection Agency under the provisions of AB2161 (Bronzan). This Risk Characterization Document addresses occupational and dietary exposure to DEF.

DEF appears to be readily absorbed by the oral route and rapidly metabolized in the species examined. The oral absorption rate was assumed to be 70% based on the average urinary excretion in rats on all dosing regimens. The dermal absorption for DEF was assumed to be 47.5% in both human and animals based on a study conducted in rats. A default assumption of 50% respiratory retention and 100% absorption was used with occupational exposure to DEF based on the assumption that DEF is primarily in the vapor phase. Several metabolic pathways have been proposed for DEF based on a few metabolites; however, the metabolism of DEF by the various routes of exposure is still highly speculative. One explanation for the inability to identify metabolites was that most of the parent compound had been extensively metabolized into natural constituents, such as fatty acids and proteins. n-Butyl mercaptan (nBM) was identified in the excreta of hens administered DEF orally. It was proposed that DEF was hydrolyzed to nBM in the gut causing the hematological effects which were only observed with oral administration of DEF. Apparently nBM inhibits glucose-6-phosphate dehydrogenase leading ultimately to red blood cell lysis through the formation of methemoglobin. nBM is thought to be a product of the normal metabolism of DEF in tissues. DEF also readily degrades to nBM in the environment and may be responsible for complaints by residents in communities near cotton fields due to its strong skunk-like odor (odor threshold ~ 0.01 to 1 ppb). However, limited data on nBM preclude a thorough toxicological evaluation.

The acute effects of DEF in experimental animals are due primarily to its inhibition of various esterases including cholinesterase (ChE) and neuropathy target esterase. The clinical signs observed include both cholinergic signs and delayed neuropathy, although delayed neuropathy was only observed in hens. Hematological changes were also seen with acute exposure to DEF. The no-observed-effect level (NOEL) for the hematological changes appears to be higher than the NOEL for the neurological effects. Although the acute studies in hens suggest that the metabolism is different by the oral route, two of the lowest acute NOELs in mammals were not significantly different after adjusting for oral or dermal absorption. Therefore, a critical NOEL of 7 mg/kg was selected to evaluate occupational and dietary exposure based on excessive salivation that was observed on pregnant female rats on day 3 of dosing at 28 mg/kg/day by oral gavage. After correcting for oral absorption, the adjusted acute NOEL was 4.9 mg/kg/day. The NOEL for dermal irritation was estimated to be 8.3 mg formulation/cm^2.

The neurological effects were also the predominant adverse effects seen with subchronic exposure, although hematological changes (reduced erythrocyte count, hemoglobin and hematocrit), some ocular effects (reduced electroretinographic (ERG) responses and pale retinal fundus) and fatty droplets in the adrenal gland were observed in a rat inhalation study. In a rat reproductive toxicity study, several reproductive effects were observed including a reduction in fertility, birth, and viability indices, an increase gestation length, reduced pup weights, cannibalism of pups, and discolored pup livers. However, the neurological effects were the most sensitive endpoints with subchronic exposure. The critical subchronic NOEL for evaluating seasonal occupational exposure was 2.0 mg/kg based on muscle fasciculations, brain ChE inhibition (85% of controls) and microscopic lesions in the skin of rabbits exposed for 3 weeks by the dermal route. After correcting for dermal absorption, the adjusted subchronic NOEL was 0.95 mg/kg/day.

Hematological changes, brain ChE inhibition, reduced weight gain, and transient hypothermia were observed in laboratory animals with chronic exposure to DEF. Hematological changes were seen in mice, rats, and dogs. Significant brain ChE inhibition was detected in rats and mice. The reduced weight gain and hypothermia were only observed in rats. In mice, there were dose-related increases in numerous non-neoplastic lesions in the gastrointestinal tract (small intestine vacuolar degeneration, dilated/distended small intestine and cecum, rectal necrosis/ulceration), liver (hypertrophy), adrenal glands (degeneration/pigmentation), and spleen (hematopoiesis). Dose-related increases in several pre-neoplastic lesions were also seen in the small intestine (mucosal hyperplasia and focal atypia) and lungs (focal hyperplasia and epithelialization). Histological changes in the small intestine (hyperplasia and vacuolar degeneration), liver (cytoplasmic vacuolation), and adrenal glands (vacuolar degeneration) were also observed in rats. In addition, numerous ocular effects were observed in one rat study including corneal opacity, lens opacity, cataracts, corneal neovascularization, iritis, uveitis, bilateral flat ERG responses, bilateral retinal atrophy, and optical nerve atrophy. The corneal opacity, cataracts, and optic nerve atrophy appear to be secondary to the degenerative changes in the retina. The critical NOEL for evaluating chronic dietary exposure to DEF was 0.2 mg/kg/day based on hematological changes and microscopic lesions in the small intestine of rats exposed to DEF in the feed for 2 years. After correcting for oral absorption (70%), the adjusted chronic NOEL was 0.14 mg/kg/day.

There was a dose-related increase in adenocarcinomas of the small intestine of both sexes, hemangiosarcomas in the liver of males, and alveolar/bronchiolar adenomas in females in a 90-week mouse feeding study. There were no dose-related increases in tumors in two rat chronic feeding studies and all the genetic toxicity studies for DEF were negative. Because the increase in tumors occurred in both sexes of mice at multiple sites (one of which was rare), a quantitative assessment of the oncogenic potency was conducted based on the incidence of hemangiosarcomas in male mice. The estimated oncogenic potency of DEF ranged from 3.3×10^{-2} to 5.8×10^{-2} $(mg/kg/day)^{-1}$.

Daily, seasonal, and lifetime exposure dosages were estimated for 9 different job categories of pesticide workers potentially exposed to DEF. An annual exposure dosage was not calculated for occupational exposure since exposure was clearly limited to a few months during the year. Five of these job categories are handlers (aerial mixer/loaders, pilots, flaggers, ground mixer/loaders and ground applicators) and four are harvesters (picker operator, module builder operator, raker, and tramper). The estimated mean absorbed daily dosages (ADDs) for workers ranged from 7.6 µg/kg/day for ground applicators to 121.8 µg/kg/day for trampers. The highest dermal exposure to DEF was to the hands and ranged from 0.20 mg formulation/cm^2 for ground applicators to 5.48 µg formulation/cm^2 for pilots. Assuming the pesticide workers were

exposed for an average of 21 days during a 45-day-day use season, the seasonal average daily dosages (SADDs) ranged from 2.1 µg/kg/day for ground applicators to 56.8 µg/kg/day for trampers. Assuming a worker is exposed for 40 years of a 70-year lifespan, the lifetime average daily dosages (LADDs) ranged from 0.1 µg/kg/day for ground applicators to 4.0 µg/kg/day for trampers. Combined occupational, dietary and ambient air exposure was initially evaluated for workers. However, the dietary and ambient air exposure was minor for most pesticide workers when compared to their occupational exposure (0.4 to 2.0% for acute exposure, 0.2 to 0.8 for seasonal exposure, and 1.3 to 6.4% for chronic exposure), so no further analysis was performed.

Dietary exposure to DEF may occur from the consumption of cottonseed products, such as cottonseed oil or cottonseed meal, or from consumption of meat or milk from livestock that were fed cottonseed products or gin trash in their feed. The potential dietary exposure was estimated for various population subgroups using anticipated residues derived from residues on whole cottonseed. The ADDs ranged from 249 to 985 ng/kg/day for the different population subgroups. The Annual Average Daily Dosages (AADDs) ranged from 13 to 103 ng/kg/day. Children, 1 to 6 years old, had the highest potential acute and chronic dietary exposure to DEF. Combined dietary and ambient air exposure was evaluated for the general population. The ADDs for combined exposure ranged from 397 to 1,288 ng/kg/day. The AADDs for combined exposure ranged from 34 to 123 ng/kg/day. Children, 1 to 6 years old, also had the highest combined exposure to DEF in the diet and ambient air.

The risk for non-oncogenic health effects in humans is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from experimental animal studies to the human exposure dosage. Generally, a margin of exposure greater than 100 is considered protective of human health when it is calculated from a NOEL derived from an animal study. The MOEs for acute neurological effects with occupational exposure ranged from approximately 40 for trampers to 1,100 for ground applicators. The MOEs for dermal irritation ranged from 1,500 to 37,000. The seasonal MOEs ranged from 17 to 86 for most pesticide workers, except ground applicators whose MOEs were greater than 400. The estimated oncogenic risk from occupational exposure to DEF was approximately 10^{-4} for most pesticide workers, except for ground applicators whose oncogenic risk was approximately 10^{-5}.

The MOEs for acute dietary exposure to DEF in the various population subgroups ranged from 5,000 to 20,000 based on anticipated residues in cottonseed products. The MOEs for the chronic dietary exposure ranged from 1,400 to 10,000. The MOEs for combined acute dietary and ambient air exposure to DEF ranged from 3,800 to 12,000. The MOEs for combined chronic dietary exposure ranged from 1,100 to 4,200. The estimated oncogenic risk from dietary exposure to DEF was between 10^{-5} and 10^{-6} for the U.S. population. However, the dietary exposure is based entirely on anticipated residues that were estimated from whole cottonseed using processing and distribution factors. The chronic dietary exposure may have been overestimated due to several assumptions including not adjusting the residue levels in cottonseed oil for the deodorization process, assuming cattle consumed cottonseed by-products in their feed at the maximum allowable level on a long-term basis, and not correcting for the percent of crop treated. The estimated oncogenic risk from combined dietary and ambient air exposure was also between 10^{-5} and 10^{-6}.

A tolerance assessment for DEF was conducted assuming commodities were consumed at the tolerance levels. After adjusting for oral absorption (70%), the estimated acute dietary intakes for various population subgroups ranged from 432 to 1,486 ng/kg/day. The resultant MOEs ranged from 3,300 to 11,000. Chronic consumption of commodities containing DEF

residues at the tolerance level was considered highly improbable based on the small percentage of samples (<1%) that had residues at or above tolerance in the DPR and California Department of Food and Agriculture pesticide monitoring programs. Therefore, a tolerance assessment for chronic dietary exposure to DEF was not conducted.

II. INTRODUCTION

A. CHEMICAL IDENTIFICATION

S,S,S-Tributyl phosphorotrithioate (DEF) is an organophosphate chemical used as a cotton defoliant. DEF induces early leaf abscission through changes in the levels of plant hormones (Ware, 1978). Defoliation occurs 4 to 7 days after treatment.

The toxicity of DEF to animals is primarily due to its inhibition of various esterases, including acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), neuropathic target esterase (NTE), and carboxylesterase. AChE is also called specific or true cholinesterase and is found near cholinergic synapses, in some organs (e.g., lung, spleen, gray matter) and in erythrocytes (Lefkowitz *et al.*, 1990). Normally, AChE metabolizes acetylcholine to acetate and choline, which results in the termination of stimulation to dendritic nerve endings and motor endplates. Acetylcholine is the neuro-chemical transmitter at endings of postganglionic parasympathetic nerve fibers, somatic motor nerves to skeletal muscle, preganglionic fibers of both parasympathetic and sympathetic nerves, and certain synapses in the central nervous system (Murphy, 1986).

Butyrylcholinesterase (BuChE), sometimes referred to as plasma cholinesterase (ChE), pseudo-cholinesterase, or serum esterase, is also inhibited by DEF. Any reference in this document to "cholinesterase", without specifically indicating that the enzyme is serum or plasma cholinesterase (ChE), should be interpreted as acetylcholinesterase (AChE). BuChE only occurs to a limited extent in neuronal elements of the central and peripheral nervous systems. In addition to plasma, it is also present in the liver, lung and other organs, although its physiological function is unknown (Lefkowitz *et al.*, 1990; Brimijoin, 1992; U.S. EPA, 1993; Pantuck, 1993). An atypical genetic variant of plasma cholinesterase has been associated with an increased susceptibility to various drugs, such as succinylcholine and cocaine (Lockridge, 1990; Pantuck, 1993). However, it is unclear if this increased susceptibility to certain drugs in people with the atypical plasma ChE translates to a possible adverse effect when plasma ChE is inhibited by organophosphates. In an *in vitro* study, it was shown that the atypical and normal plasma ChE was equally sensitive to the organophosphate inhibitors, diisopropylfluoro-phosphonate (DFP) and tetraethylpyrophosphonate (TEPP), but the atypical plasma ChE was less sensitive than the normal plasma ChE to 14 drugs, especially succinylcholine and decamethonium (Kalow and Davis, 1958). In another study, rats that were depleted of plasma AChE by injecting them intravenously with antibodies specific to this enzyme were not more susceptible to paraoxon toxicity than untreated controls based on their performance in a functional observational battery and AChE activity in the brain and diaphragm (Padilla et al., 1992). The investigators concluded that based on the results with plasma AChE, it was unlikely that plasma BuChE or erythrocyte AChE would offer significant protection against paraoxon toxicity, either.

The inhibition of AChE results in the accumulation of endogenous acetylcholine in nerve tissue and effector organs. In acutely toxic episodes, muscarinic, nicotinic and central nervous system (CNS) receptors are stimulated with characteristic signs and symptoms occurring throughout the peripheral and central nervous systems (Ellenhorn and Barceloux, 1988; Murphy, 1986). Muscarinic effects can include increased intestinal motility, bronchial constriction and increased bronchial secretions, bladder contraction, miosis, secretory gland stimulation and bradycardia. Nicotinic effects include muscle weakness, twitching, cramps and general fasciculations. Accumulation of acetylcholine in the CNS can cause headache,

A. CHEMICAL IDENTIFICATION (cont.)

restlessness, insomnia, anxiety and other non-specific symptoms. Severe poisoning results in slurred speech, tremors, ataxia, convulsions, depression of respiratory and circulatory centers and, eventually, coma.

NTE inhibition in the hen brain of greater than 70% is associated with the organophosphate-induced delayed neuropathy (OPIDN) produced by some organophosphate compounds with acute exposure (Carrington, 1989; Abou-Donia and Lapadula, 1990). Slightly lower levels of inhibition (~50%) are needed with chronic exposure. The physiological or biochemical role of NTE is unknown at this time. Aging of the phosphorylated enzyme (loss of an alkyl group) apparently is also important in the induction of this neuropathy.

Carboxylesterase is involved in the detoxification of various chemicals, including pesticides. Inhibition of carboxylesterase by DEF has resulted in the potentiation of organophosphate pesticides such as malathion that contain a carboxylic ester group (Murphy *et al.*, 1976). However, DEF also markedly potentiated the toxicity of azinphos-methyl which does not contain any carboxylic ester groups (Gaughan *et al.*, 1980). Inhibition of other detoxification enzymes may be involved. Inhibition of liver microsomal esterases is thought to be responsible for the potentiation of permethrin toxicity by DEF (Gaughan *et al.*, 1980). The absorption of phthalate diesters is reduced by DEF apparently due to its inhibition of esterase activity in the intestinal mucosa (White *et al.*, 1980). DEF is also a potent inhibitor of liver arylamidase *in vitro* (Satoh and DuBois, 1973).

B. REGULATORY HISTORY

DEF was first registered in 1960 for cotton defoliation and this has remained its only use (U.S. EPA, 1981). In 1981, the U.S. EPA issued a decision not to initiate a Rebuttable Presumption Against Registration (RPAR) review of DEF despite evidence of irreversible neurotoxic effects in laboratory animals exposed to DEF. This decision was based on the lack of evidence of neurotoxic symptoms among applicators exposed to DEF and adequate margins of exposure when specified protective clothing is worn.

In August 1991, the Department of Pesticide Regulation (DPR) in the California Environmental Protection Agency placed DEF in reevaluation based on inadequate acute toxicity data to assess the appropriateness of the signal words and precautionary statements on the label. Primary eye and dermal irritation studies for the technical grade material and a complete set of acute toxicity studies for the formulations were submitted by the registrants to DPR. The registrants were informed that the signal words and precautionary language on the current label were not adequate to mitigate possible eye and skin irritation hazards from the use of these products. The registrants have submitted proposed label amendments which have been approved by the U.S. EPA and are currently under review by DPR.

Section 13131 of the Food and Agricultural Code of California requires DPR to conduct an assessment of dietary risks associated with the consumption of produce and processed foods treated with pesticides. This assessment integrates data on acute health effects and the mandatory health effects studies specified in subdivision (c) of Section 13123, appropriate dietary consumption estimates, and relevant residue data to quantify consumer risk. This Risk Characterization Document addresses the potential adverse human health effects associated with both occupational and dietary exposure to DEF.

C. TECHNICAL AND PRODUCT FORMULATIONS

There are two products currently registered in California which contain S,S,S-tributyl phosphorotrithioate as the active ingredient, DEF 6 and Folex 6 EC. DEF 6 is manufactured by Miles Inc. (formerly Mobay Corporation) while Folex 6 EC is manufactured by Rhone-Poulenc. Folex used to contain S,S,S-tributyl phosphorotrithioite which is rapidly converted to S,S,S-tributyl phosphorotrithioate by oxidation within a few hours after exposure to air (Obrist and Thornton, 1978). However, it was reformulated and now contains only S,S,S-tributyl phosphorotrithioate. The concentration of S,S,S-tributyl phosphorotrithioate in these formulations is approximately 70%. The Material Safety Data Sheet (MSDS) for DEF 6 indicates that the other inert ingredients are trimethylbenzenes (20-30%), Ingredient 1923 (1-10%), xylenes (1-5%), and ethylbenzene (1-2%) (National Agricultural Chemicals Association, 1990). The inert ingredients for Folex 6 are not identified in its MSDS.

D. USAGE

The recommended application rate for DEF is approximately 1 to 2.5 pints (0.75 to 1.9 lb. active ingredient) per acre. It can be applied as a dilute spray in 5-10 gallons of water per acre by air or in 20-25 gallons of water per acre with ground equipment. It cannot be used through any type of irrigation system. DEF is applied predominantly by air in California. Under favorable conditions DEF gives effective defoliation within 4 to 7 days after application. When continued low temperatures prevail at night (< 60^{o}F), complete defoliation may require 9 to 14 days. In 1995, 883,857 lbs. of DEF were used in California in 6,572 applications over 605,188 acres (DPR, 1996a). DEF represented approximately 6 percent of the total pounds of pesticides applied to cotton fields in 1995.

The labels for these formulations require that applicators and other handlers wear the following protective clothing: coveralls over a long-sleeved shirt and long pants, chemical-resistant gloves, chemical-resistant footwear over socks, protective eyewear, chemical-resistant headgear, chemical-resistant apron when mixing/loading and cleaning equipment, and a MSHA/NIOSH-approved respirator in enclosed areas or MSHA/NIOSH-approved dust/mist filtering respirator for outdoors. According to the federal worker protection standards for agricultural pesticides, the protective clothing requirements for mixer/loaders may be reduced to work clothing (long-sleeved shirt and long pants), chemical-resistant apron, and chemical-resistant gloves when using closed mixing/loading systems. Applicators may also reduce the protective clothing to work clothing when closed systems are used. California regulations require that a closed system be used for all mixing and transfers of cotton defoliants and that there is a ½-mile buffer zone between residential areas and sprayed fields (California Code of Regulations, Title 3, Section 6470). In addition, California regulations require that the level of n-butyl mercaptan (nBM) in formulated products containing DEF cannot exceed 0.1 percent (California Code of Regulations, Title 3, Section 6361). The restricted reentry interval in areas treated with DEF is 24 hours. For all other activities involving human contact with the foliage, the reentry interval is 4 days. The minimum preharvest interval is 7 days.

E. ILLNESS REPORTS

In 1977, the California Department of Food and Agriculture (CDFA) published a report summarizing several hundred complaints it had received that were associated with DEF (Maddy and Peoples, 1977). The complaints usually involved wheezing, coughing, nausea, and other

discomforts that were attributed to the degradation product, nBM, a volatile degradation product of DEF with a strong skunk-like odor. This odor apparently can be detected by humans at air concentrations as low as 0.01 ppb (Santodonato *et al.*, 1985).

Approximately 13 drums containing DEF and Merphos were damaged on a ship in transit from Mexico to Australia (McLeod, 1975). Caustic soda was used in the process of cleaning up the damaged drums in Auckland, New Zealand, which resulted in increased liberation of nBM. Over 600 people were seen at a local hospital with various complaints. Symptoms observed in 49 cases were attributed to organophosphate poisoning (excessive salivation, sweating, muscle weakness, fatigue, nausea, vomiting, diarrhea, and miosis); however, no cholinesterase inhibition was found. Symptoms attributed to nBM (headache, dizziness, dry mouth and throat constriction) were reported in another 192 cases. It was estimated that the air levels of nBM exceeded 0.5 ppm (ACGIH TWA-TLV) and in some places exceeded 10 ppm. It was not possible to categorize the symptoms observed in the remaining cases. It was reported that panic may have been a factor in some of these cases since there had been widespread coverage of the spill in the news media.

More recently, the California Department of Health Services also did an epidemiology study in which they examined the relationship of the health symptoms and community exposure to cotton defoliants (Scarborough *et al.*, 1989). Four-hundred and six residents in six agricultural communities in the San Joaquin Valley were surveyed by phone during the time of cotton defoliation. They found a significantly greater risk for eye and throat irritation, rhinitis, fatigue, shortness of breath, nausea and diarrhea in the high exposure group (people who lived or worked within one mile of a cotton field that had been treated within the previous two weeks). In the high-exposure group, there was also a significantly greater risk for these self-reported symptoms in the subgroup noticing a strong odor, suggesting that DEF or nBM was the causative agent.

There were a total of 16 illness and injury cases associated with exposure to DEF and DEF in combination with other pesticides in California from 1982 through 1991 (DPR, 1994a). Of the 16 cases, 11 were systemic illnesses, two were eye injuries, and three were respiratory illnesses. Systemic poisoning due to exposure to DEF and DEF in combination with other pesticides was positively identified (definite) in four cases. One systemic case was classified as probable and six others as possible cause of the acute poisoning.

No cases of delayed neurotoxicity in humans have been reported for DEF, although there was one case where a 28-year-old agricultural worker spilled Merphos on his arm (Fisher, 1977). He did not develop any acute symptoms, but four days later his hands and arms became weak. He finally sought medical attention six days after exposure when he could barely move his arms or legs. After admission to the hospital, his plasma cholinesterase level was normal despite his symptoms. Eight days later, complete facial paralysis developed. Electromyography demonstrated decreased voltage of action potentials, delayed conduction velocity, increased insertional activity, and denervation potentials. Recovery was complete after fourteen weeks of intensive physical therapy.

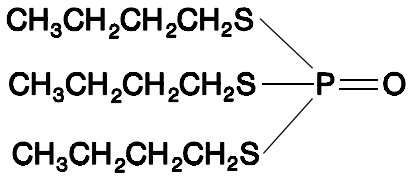
F. PHYSICAL/CHEMICAL PROPERTIES

DEF (Talbott, 1990)

1. Common Names: DEF, Tribufos, Butiphos, Merphos Oxide

F. PHYSICAL/CHEMICAL PROPERTIES (cont.)

2. Chemical Name: S,S,S-Tributyl phosphorotrithioate
3. Trade Names: DEF, Folex
4. CAS Registry No.: 78-48-8
5. Empirical Formula: $C_{12}H_{27}OPS_3$
6. Molecular Structure:



7. Molecular Weight: 314.5
8. Physical State: Colorless to yellow liquid
9. Odor: Skunk-like
10. Melting Point: < -25°C
11. Boiling Point: 150°C at 0.3 mm Hg
12. Density: 1.057 g/cm at 20°C
13. Solubility: Water - 2.3 ppm at 20°C (Leimkuehler, 1980)
    Solvents - Completely miscible with n-hexane, dichloromethane, toluene, and 2-propanol (Betker, 1985)
14. Vapor Pressure: 6.5×10^{-6} mm at 25°C (Talbott and Mosier, 1987)
15. Octanol-Water Partition Coefficient: 3.31×10^{5} at 25°C (D'Harlingue, 1987)
16. Henry's Law Constant: 2.9×10^{-7} atm x m^3/mole at 20°C (Talbott, 1987)

n-Butyl Mercaptan (ACGIH, 1986)

1. CAS Registry No.: 109-79-5
2. Empirical Formula: $C_4H_{10}S$
3. Molecular Structure: **$CH_3CH_2CH_2CH_2SH$**

F. PHYSICAL/CHEMICAL PROPERTIES (cont.)

4. Molecular Weight: 90.19

5. Density: 0.83679 at 25^{o}C

6. Melting Point: -115.9^{o}C

7. Boiling Point: 97.2 – 101.7^{o}C

8. Vapor Pressure: 83 mg Hg at 25^{o}C

9. Solubility: Slightly soluble in water, but very soluble in alcohols, ether, and liquid hydrogen sulfide

G. ENVIRONMENTAL FATE

Field Dissipation

DEF was applied once to soil at 3.375 lb. a.i./acre in two different sites in California, one near Fresno and one near Watsonville (Grace and Cain, 1990). Soil samples were analyzed for DEF and its metabolite, dibutyldisulfide. None of the samples contained $\geq$ 0.01 ppm dibutyldisulfide. Only two samples had DEF more than 6 inches below the surface and DEF in these two samples was still less than 12 inches below the surface. The half-lives were 15.3 and 47.7 days for the Watsonville and Fresno sites, respectively.

Hydrolysis

DEF was relatively stable in aqueous solutions at pH 5 and 7 up to 32 days (94.5% and 94.6% recovered, respectively), but degraded slightly at pH 9 (80.8% recovered after 32 days) (Schocken and Philippson, 1987). The half-life at pH 9 was estimated to be 124 days. The polar breakdown product was identified as desbutylthio DEF.

Photolysis

DEF was stable in a sandy loam soil exposed to natural sunlight for 30 days (Jackson *et al.*, 1988); however, it degraded in aqueous solutions (pH 7) exposed to natural sunlight for 30 days (Kesterson and Lawrence, 1990). The estimated half-life was 44 days. No photodegradation products were identified.

Soil Adsorption and Mobility

The soil adsorption coefficient (K_d) and constant (K_{oc}) were determined for DEF with four different soil types (sand, sandy loam, silty loam, and clay loam) (Daly, 1987). The estimated K_d values ranged from 60.6 for sandy loam soil to 106 for clay loam. The estimated K_{oc} values ranged from 4,870 for silt loam to 12,684 for sand. In a column leaching study, DEF was applied to the top of columns (1.6 cm by 45 cm) containing clay loam, sandy loam, loam or muck soil (Church and Shaw, 1969). DEF remained in the top 4 cm of soil regardless of soil type. DEF was detected only in the leachate of the sandy loam soil at less than 1% of the

G. ENVIRONMENTAL FATE (cont.)

applied dose. In another study, ^{14}C-DEF was incubated aerobically in sandy loam soil at room temperature for 32 days (aged) and then applied to the top of a column (5.4 cm by 45 cm) containing sandy loam soil (Schocken and Parker, 1987). The vast majority (94.7%) of the applied radioactivity was found in the upper 6 cm of the soil columns with most (74.7%) of this radioactivity identified as the parent compound. Less than 1% of the applied radioactivity was found in the leachate.

Soil Metabolism

The estimated half-life for DEF was 198 days when incubated with sandy loam soil in the dark under aerobic conditions (Olson *et al.*, 1990). The half-life of DEF under anaerobic conditions was 64.8 days (Olson *et al.*, 1989). However, in both studies the material balance was less than 50%, so interpretation of the results is difficult.

Summary

The water solubility, soil adsorption, hydrolysis and aerobic soil metabolism data suggest that DEF is not likely to be a ground water contaminant. DEF may become airborne from drift after aerial application or ground spraying. DEF is not a very volatile compound based on its vapor pressure; however, the degradation product, nBM, is volatile with a skunk-like odor and is probably responsible for a number of complaints in areas near where DEF has been applied.

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

Summary: DEF appears to be readily absorbed by the oral route and rapidly metabolized in the species examined. The oral absorption was assumed to be 70% based on the average urinary excretion in rats on all dosing regimens. The dermal absorption for DEF was assumed to be 47.5% in both human and animals based on a study conducted in rats. A default assumption of 50% respiratory retention and 100% absorption was used with DEF based on the assumption that DEF is primarily in the vapor phase with occupational exposure.

Absorption

[^{14}C] DEF was administered to 5 rats/sex/dose in a single dose by oral gavage at 5 or 100 mg/kg or in 14 consecutive doses at 5 mg/kg/day (Kao *et al.*, 1991). Approximately 95-98% of the total dose was excreted in the urine and feces within 72 hours after dosing. Most of the radioactivity was excreted within 24 hours after a single dose at 5 mg/kg (M:91%;F:87%) or 100 mg/kg (M:75%;F:57%). A similar percentage (M:89%;F:85%) was excreted within 24 hours after 14 consecutive doses at 5 mg/kg/day. The majority of the radioactivity was excreted in the urine after a single dose at 5 mg/kg (M:55%;F:66%) or 100 mg/kg (M:60%;F:70%). A slightly higher percentage (M:73%;F:80%) was excreted in the urine after 14 consecutive doses at 5 mg/kg/day.

Male rats had [^{14}C] DEF (98.9% - mixed in distilled water with DEF 6 blank formulation) applied to their shaved backs at 1.93, 12.4 and 100 µg/cm^2 for 10 hours (Schroeder, 1992). The application site was protected by a non-occlusive cover of a Teflon-laminated filter and a carbon-impregnated material. Four rats/dose were sacrificed at 1, 4, 10 and 168 hours (7 days). The amount excreted in the urine over 7 days ranged from 25.8 to 36.0% of the applied dose decreasing from the low to high dose level. On the other hand, the amount excreted in the feces was fairly similar (3.2 to 3.6% of applied dose) at the different dose levels. After correcting for recoveries, the mean dermal absorption rates were 47.5, 47.9 and 33.9% at 1.93, 12.4, and 100 µg/cm^2, respectively. The dermal absorption rate at the lowest concentration was selected for use in exposure calculations since it is closest to the concentration of DEF on the skin of workers.

The pharmacokinetics of DEF after intravenous administration was not studied due to the low water solubility of DEF. However, based on the nearly complete elimination of DEF by the urinary route when applied dermally to rats (Schroeder, 1992), it was assumed the amount excreted by the biliary route is insignificant when DEF is administered orally. Based on this finding, DPR assumed that with oral administration most of the radioactivity in the feces was unabsorbed material. Therefore, the oral absorption rate for DEF was estimated to be 70% based on the approximate average urinary excretion for all dosing regimens.

There were no data available on the absorption of DEF by the inhalation route.

Distribution

In laying hens administered DEF at 50 mg/kg by the oral and dermal routes, the half-lives were 2.7 and 3.8 days, respectively, based on the plasma concentration curve (Abou-Donia *et al.*, 1984). Hall (1991) measured residues in the liver, fat, muscle, and eggs of 6 laying

hens given [^{14}C] DEF and 4 laying hens given [^{35}S] DEF at 4 mg/kg/day in gelatin capsules for 3 consecutive days. Four hours after the last dose, the highest residues were found in liver followed by internal eggs, muscle, and fat.

Tissue residues were also determined in two lactating goats 21 hours after receiving [^{14}C] DEF at 0.82 or 0.85 mg/kg/day in gelatin capsules for 3 consecutive days (Sahali, 1991). Of the four tissues examined (muscle, fat, kidney, liver), the liver had the highest residues and the muscle had the lowest. The residues in milk were between those of fat and muscle.

The most extensive residue analysis of tissues was conducted by Kao *et al.* (1991) in rats administered [^{14}C] DEF by oral gavage at 5 or 100 mg/kg. Less than 3% of the total dose was found in the tissues and carcasses 72 hours after dosing. The highest residue levels were found in the liver, followed by fat, lung, kidney, blood, gastrointestinal tract, spleen, bone, heart, gonads, muscle, and brain.

Biotransformation

One of the initial steps in the metabolism of DEF appears to be its oxidation to an active metabolite, such as a sulfoxide. DEF was converted to a more potent ChE inhibitor with the addition of microsomal fractions or purified cytochrome P-450 isozymes from mouse liver *in vitro* (Wing *et al.*, 1984; Levi and Hodgson, 1985). Hall (1991) and Sahali (1991) were unable to identify any of the metabolites in the tissues analyzed from laying hens and goats, respectively. Based on the complexity of the metabolite profiles and the extreme polarity of many of the metabolites in these tissues, these investigators suggested that most of the parent compound had been incorporated into natural constituents. Radioactive residues were detected in fatty acids and proteins in goat tissues (Sahali, 1991).

Kao *et al.* (1991) detected over 18 radioactive metabolites in the urine of rats, but only one was identified, butyl-gamma-glutamylcysteinylglycine disulfide. In feces, the parent compound and an unidentified non-polar metabolite accounted for 15 to 31% and 1% of the total dose, respectively. They proposed a metabolic pathway for DEF, which involves the initial hydrolysis of DEF to S,S-dibutyl phosphorodithioate and nBM (Figure 1). nBM is converted to the fatty acid, butyric acid, which may be further metabolized through the usual metabolic pathways for fatty acids. S,S-Dibutyl phosphorodithioate is further metabolized to nBM and phosphate. Hur and coworkers (1992) proposed a similar metabolic pathway based on the isolation of two metabolites (S,S-dibutyl phosphorodithioate and S,S-dibutyl phosphorothioic acid) after incubation of DEF with mouse liver microsomes and in rat urine after intraperitoneal injection of DEF at 100 mg/kg. They proposed that these metabolites were formed via DEF sulfoxide and S,S-dibutyl,S-1-hydroxybutyl phosphorotrithioate, respectively, which are reactive intermediates formed by microsomal mixed function oxidases (MFOs), such as cytochrome P-450.

Abou-Donia and coworkers isolated nBM in the plasma and excreta of hens administered a single oral dose of DEF at 400 mg/kg or 30 daily oral doses at 20-80 mg/kg/day (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators concluded that a portion of orally administered DEF is converted to nBM in the gastrointestinal tract through hydrolysis. These studies are discussed in more detail in the Neurotoxicity Section of the Toxicology Profile.

A. PHARMACOKINETICS (cont.)

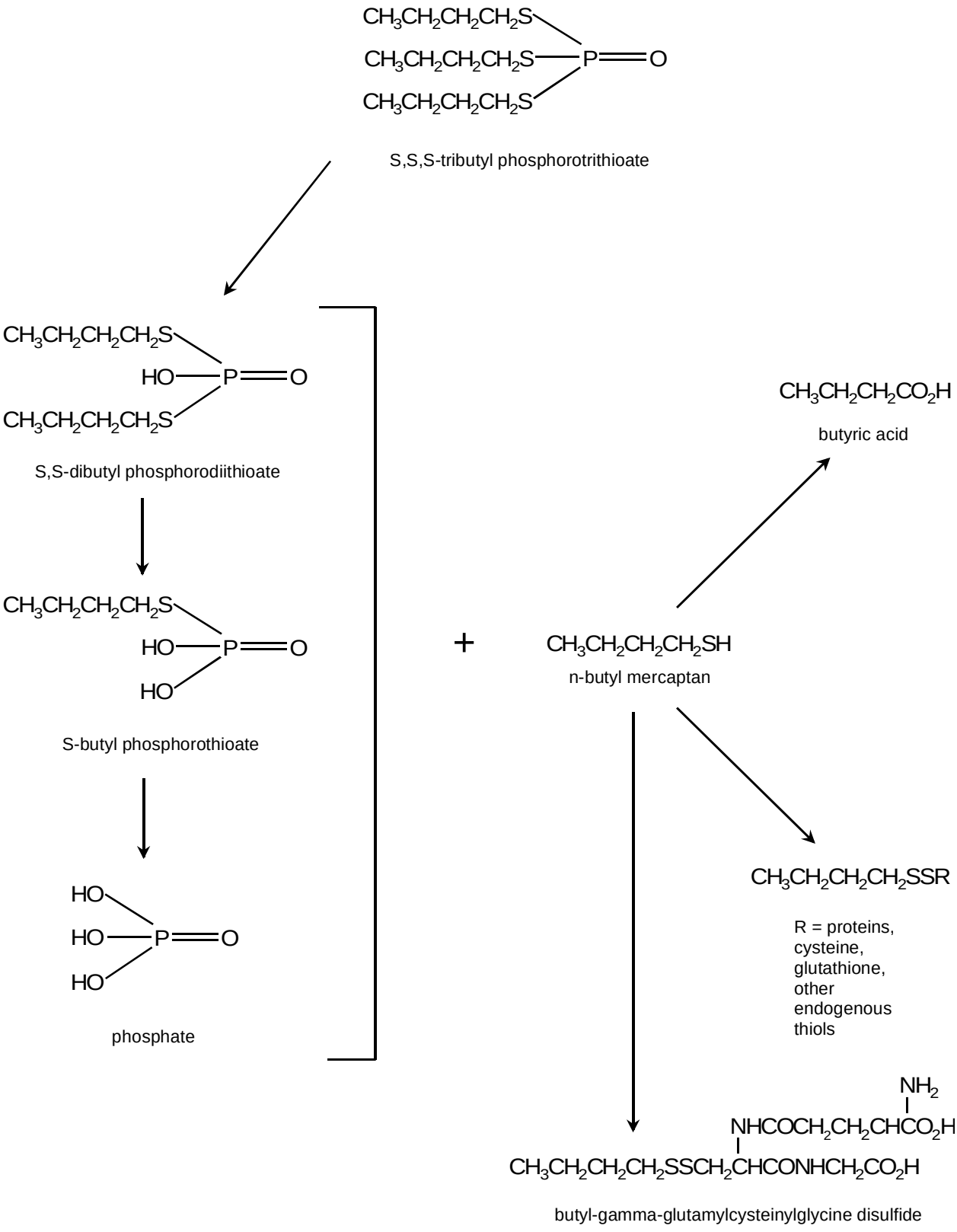


Figure 1. Proposed Metabolic Pathway for DEF (Kao *et al.*, 1991)

A. PHARMACOKINETICS (cont.)

Excretion

As mentioned previously under absorption, the major route of excretion in rats was the urinary route with an average excretion rate at 72 hours between 55 to 80% (*Kao et al.*, 1991). In the first 24 hours, the urinary excretion rate was affected by both dosage (decreased with increasing dosage) and sex (lower in males). The average amount excreted in the urine at 24 hours was lowest (M:44%;F:40%) in rats administered a single dose at 100 mg/kg; however, by 72 hours the total amount excreted in the urine was similar (M:60%;F:70%) to rats administered a single dose at 5 mg/kg (M:55%;F:66%). The urinary excretion was highest after administration of DEF at 5 mg/kg/day for 14 consecutive days at both 24 hrs (M:67%;F:72%) and 72 hrs (M:73%;F:80%), suggesting more efficient absorption and/or metabolism with continued exposure. The average urinary excretion was higher in females on all of the dosing regimens at 72 hours (M:55-73%; F:66-80%), suggesting the absorption and/or metabolism of DEF is more efficient in females than males. A significant amount of DEF was also eliminated in the feces of rats within 72 hours following a single oral dose of DEF at 5 mg/kg (M:42%;F:30%) or 100 mg/kg (M:38%;F:27%). The fecal excretion was slightly lower after 14 consecutive doses at 5 mg/kg/day (M:24%;F:15%). Only 1% was excreted as CO_2 in expired air for either sex.

B. ACUTE TOXICITY

Summary: The standard battery of acute toxicity tests was available for both the technical grade DEF and the formulations. Nine of the 17 available acute toxicity tests were acceptable based on FIFRA guidelines. The clinical signs observed in animals after acute exposure to DEF were typical cholinergic signs (e.g., ataxia, tremors, facial and urogenital stains). With inhalation exposure, dyspnea, red turbinates, and firm zones in the lungs were also reported. Erythema was also observed with dermal exposure. When comparing LD_{50}/LC_{50} values for the different routes in animals, technical grade DEF appears to be slightly more toxic by the inhalation route than the oral route and least toxic by the dermal route. The higher dermal LD_{50} values also suggest that absorption of DEF by the dermal route is slower or incomplete. For technical grade DEF, the lowest-observed-effect level (LOEL) in an acceptable inhalation LC_{50} study with rats was 1,590 mg/m^3 (254 mg/kg) based on death, cholinergic signs, red turbinates and "firm zones." In an acceptable rat oral LD_{50} study with rats, the LOEL was 192 mg/kg based on cholinergic signs. The LOEL in an acceptable dermal LD_{50} study with rabbits was 500 mg/kg based on cholinergic signs and erythema. No-observed-effect levels (NOELs) were not established in any of the acceptable acute studies for technical grade DEF. Acute effects observed in subchronic, developmental, and neurotoxicity studies are not included here, but are discussed later under those sections. All acute effects are summarized under Acute Toxicity in the Hazard Identification section.

Several acute toxicity tests were also conducted on the degradation product, nBM, and the metabolite, 3-hydroxybutylmethyl sulfone; however, none of these tests met FIFRA guidelines. The effects observed in animals exposed to nBM were typical of CNS depression. The pathological findings included kidney and liver damage with all routes of exposure and lung damage with inhalation exposure. Ocular irritation was also observed. A comparison of LC_{50}/LD_{50} values suggests that nBM is less acutely toxic than DEF. LOELs and NOELs could not be established in any acute toxicity studies for nBM.

B. ACUTE TOXICITY (cont.)

Technical Grade DEF

The acute toxicity of technical grade DEF is summarized in Table 1. In one acute inhalation study with technical grade DEF, the LOEL was 2,920 and 1,590 mg/m^3 (234 and 127 mg/kg)[1] for male and female rats, respectively with a 4-hour, nose-only exposure (Warren, 1990). Analysis of particle size indicated that the mass mean aerodynamic diameter was approximately 1.5 µm and that greater than 87% of the particles were less than 2 µm. Based on the particle size analysis, DPR assumed that 100% of DEF in the inhaled air reached the alveoli and was absorbed. Unthriftiness, hypoactivity, urine stains, nasal discharge, red eye discharge, lacrimation, ataxia, tremors, death, excitability, vocalization, dyspnea, red turbinates, and firm zones in the lungs were observed at the LOEL. A NOEL was not established in this study, although it was acceptable based on the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines. In another 4-hour (nose only) acute inhalation study in rats, a NOEL was established at 77 mg/m^3 (6.2 mg/kg)[1] based on abnormal behavior, including decreased preening and lethargy (Thyssen, 1978a). However, this study had several deficiencies including no summary of clinical signs by dose group, no gross necropsy, and no analysis of particle size.

In an acceptable acute oral toxicity study, death occurred at 235 and 429 mg/kg and higher in female and male rats, respectively (Sheets, 1991a). The NOEL was less than 192 mg/kg for females and 294 mg/kg for males based on urine stain (M:3/5,F:4/5), red lacrimal stain (M:4/5,F:2/5), clear lacrimation (M:3/5,F:3/5), diarrhea (M:0/5,F:5/5), perianal stains (M:1/5,F:4/5), red nasal stain (M:4/5,F:0.5), decreased activity (M:2/5,F:0/5), salivation (M:1/5,F:0/5), dyspnea (M:1/5,F:0/5), wheezing (M:1/5,F:0/5), and clear nasal stain (M:1/5,F:0/5).

After dermal administration, deaths were observed at 1,000 mg/kg and higher in rabbits (Sheets and Phillips, 1991). The NOEL was less than 500 mg/kg based on tremors (1/10), muscle fasciculations (10/10), erythema at the site of application (9/10), hypoactivity (2/10), clear nasal discharge (2/10), white nasal discharge (1/10), ataxia (1/10), increased reactivity (1/10), clear lacrimation (1/10), and clear lacrimal stain (1/10). This study was also acceptable based on FIFRA guidelines.

Technical grade DEF was only mildly irritating to the skin and eyes of rabbits (Crawford and Anderson, 1972a; Sheets and Fuss, 1991; Sheets and Phillips, 1992a). DEF did not induce a sensitization response in guinea pigs using the Buehler patch test (Sheets, 1990).

At environmental temperatures below 30^{o}C, hypothermia has been observed in rats, mice, and guinea pigs, but not rabbits after a single dose of DEF between 20 and 200 mg/kg by the oral, intraperitoneal or intravenous route (Ray, 1980; Ray and Cunningham, 1985). At doses greater than 100 mg/kg, the hypothermia persisted for several days. The hypothermia was associated with piloerection, sluggishness, and irritability, but a high degree of motor control even when body temperature reached 30^{o}C. As body temperatures dropped below 30^{o}C, deaths occurred usually after prolonged hypothermia. The hypothermia appears to be due to a block of shivering and non-shivering thermogenesis with little effect on basal metabolism, heat conservation or motor control. The investigators suggested a selective action

[1] Estimated assuming a respiratory rate of 0.16 m^3/kg/4 hrs for a rat (Zielhuis and van der Kreek, 1979).

B. ACUTE TOXICITY (cont.)

Table 1. The Acute Toxicity of Technical Grade DEF (95-99.7%)

Species	Sex	Results	References[a]
		Acute Inhalation LC_{50}	
Rat	M	4,000 mg/m^3 (4-hr, nose only)	1
	F	1,600 mg/m^3 (4-hr, nose only)	
	M	4,650 mg/m^3 (4-hr, nose only)	2*
	F	2,460 mg/m^3 (4-hr, nose only)	
		Acute Oral LD_{50}	
Rat	M	435 mg/kg	3*
	F	234 mg/kg	
		Acute Dermal LD_{50}	
Rabbit	M/F	1,093 mg/kg	4*
		Primary Dermal Irritation	
Rabbit	M/F	Mild Irritant	5,6*
		Primary Eye Irritation	
Rabbit	M/F	Mild Irritant	5,7*
		Dermal Sensitization	
Guinea Pig	M/F	Non-Sensitizer	8

[a] References: 1. Thyssen, 1978a; 2. Warren, 1990; 3. Sheets, 1991a; 4. Sheets and Phillips, 1991; 5. Crawford and Anderson, 1972a; 6. Sheets and Fuss, 1991; 7. Sheets and Phillips, 1992a; 8. Sheets, 1990.
* Acceptable study based on the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines

on a central thermogenic control process may be involved. Other research indicates that the hypothermia associated with organophosphates is due to central AChE inhibition because it is antagonized by centrally active antiChE drugs, such as atropine, but not by peripherally active antiChE drugs, such as 2-PAM (Kenley *et al.*, 1982). A NOEL could not be established for this effect from these studies, although the effect was minimal with intraperitoneal injection of DEF at 20 mg/kg.

DEF Emulsifiable Concentrates

The acute toxicity of DEF emulsifiable concentrates is summarized in Table 2. The signs observed with DEF emulsifiable concentrates were similar to those observed with technical DEF. With inhalation exposure in rats, the LOEL was 540 mg formulation/m^3 (4-hr, nose-only) (Warren and Tran, 1992). Hypoactivity, lacrimation, red nasal discharge, and unthriftiness were observed at this dose, but no mortalities or gross lesions. Red lungs and nasal turbinates were observed at necropsy at higher doses. A NOEL was not established for this study. The lowest LOEL by the oral route was 290 mg formulation/kg in female rats

B. ACUTE TOXICITY (cont.)

Table 2. The Acute Toxicity of DEF Emulsifiable Concentrates (70%)

Species	Sex	Results	References[a]
		Acute Inhalation LC_{50}	
Rat	M	> 1,350 mg/m^3 (1-hr)	1
	F	> 1,450 mg/m^3	
	M	3,550 mg/m^3 (4-hr, nose only)	2*
	F	2,340 mg/m^3	
Mice	M	2,120 mg/m^3 (30-min)	3
		Acute Oral LD_{50}	
Rat	M	570-712 mg/kg	4,5*
	F	349 mg/kg	
		Acute Dermal LD_{50}	
Rabbit	M/F	300 mg/kg	6
Rat	M/F	> 2,000 mg/kg	7*
		Primary Dermal Irritation	
Rabbit	M/F	Corrosive	8,9*
		Primary Eye Irritation	
Rabbit	M/F	Severe Irritant	8

[a] References: 1. Kimmerle, 1972; 2. Warren and Tran, 1992; 3. DuBois and Meskauskas, 1968; 4. Crawford and Anderson, 1972b; 5. Sheets and Phillips, 1992b; 6. Crawford and Anderson, 1972c; 7. Astroff and Phillips, 1992a; 8. Crawford, 1971; 9. Sheets and Phillips, 1992c.

* Acceptable study based on the FIFRA guidelines.

(Sheets and Phillips, 1992b). The effects reported at the LOEL were tremors, hypoactivity, increased reactivity, vocalizations, hunched back, labored breathing, muscle fasciculations, lacrimation, nasal, ocular, oral and perianal stains. Compound-related gross lesions in animals that died included discolored stomach zones, red fluid in the bladder, gas/fluid in the intestines, and fluid in the abdomen. There appears to be a species difference in sensitivity based on the dermal LD_{50} values for rabbits and rats (300 vs. >2,000 mg formulation/kg, respectively); however, the NOELs appear to be similar. With rabbits, a NOEL was established at 106 mg formulation/kg based on unspecified cholinergic signs (Crawford and Anderson, 1972c). In rats, a NOEL was not established; however, the LOEL was 500 mg formulation/kg in both sexes of rats based on ataxia, increased reactivity, irritation at application site, and nasal and perianal stains (Astroff and Phillips, 1992a). No mortalities or gross lesions were seen at the LOEL. A DEF emulsifiable concentrate produced severe skin and eye irritation in rabbits (Crawford, 1971; Sheets and Phillips, 1992c) which appears to be due primarily to the inert ingredients since the technical grade DEF was only mildly irritating (Crawford and Anderson, 1972a). No studies were available on the dermal sensitization potential of the DEF emulsifiable concentrates.

B. ACUTE TOXICITY (cont.)

N-Butyl Mercaptan

The acute toxicity of nBM, a major degradation product of DEF, was examined by one laboratory (Table 3) (Fairchild and Stokinger, 1958). Based on the LD_{50} and LC_{50} values, nBM appears to be less toxic than DEF. The inhalation LC_{50} estimates for nBM ranged from 2,500-4,020 ppm (9,202-14,798 mg/m^3) which were significantly higher than those for DEF which ranged from 1,600-4,650 mg/m^3. The oral LD_{50} estimated for nBM (1,500 mg/kg) was also significantly higher than those for DEF (234-435 mg/kg). The clinical signs observed after exposure to nBM were indicative of CNS depression. The signs observed in approximate order of appearance included restlessness, increased respiration, incoordination, muscular weakness, skeletal muscle paralysis, cyanosis, lethargy, sedation, respiratory depression, coma, and death. The signs were similar regardless of the route of exposure, except that with oral exposure, where diarrhea was also observed and with inhalation exposure, where watery eyes and sneezing were also observed. nBM also produced slight irritation in an ocular irritation test with rabbits. The pathological findings with all routes of exposure included indications of kidney damage (cloudy swelling of the tubules and hyaline casts in the lumina) and liver damage (lymphocytic infiltration and necrotic foci with small hemorrhages). With inhalation exposure, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage in the lung were also observed. The systemic and local toxicity after exposure to nBM by the dermal route was not examined. The lungs appear to be an important route of excretion for nBM because a strong odor was detected in the expired air of animals regardless of the route of exposure. It was not possible to establish a NOEL by any route since the incidences of clinical signs and pathological lesions were not summarized by dose level.

Table 3. The Acute Toxicity of Technical Grade n-Butyl Mercaptan

Species	Sex	Results	Reference[a]
		Acute Inhalation LC_{50}	
Rat	M	4,020 ppm (4-hr)	1
Mice	M	2,500 ppm	
		Acute Oral LD_{50}	
Rat	M	1,500 mg/kg	1
		Acute Intraperitoneal LD_{50}	
Rat	M	399 mg/kg	1
		Primary Eye Irritation	
Rabbit	M	Slight Irritant	1

[a] Reference: 1. Fairchild and Stokinger, 1958.

3-Hydroxybutylmethyl Sulfone Metabolite

In an acute oral toxicity study, 5 female Sprague-Dawley rats were given 3-hydroxybutylmethyl sulfone in water by gavage at 0 or 2,000 mg/kg (Astroff and Phillips, 1992b). There were no mortalities, reduction in body weights or treatment-related gross lesions. Ataxia,

B. ACUTE TOXICITY (cont.)

lacrimation, hypoactivity, hyperactivity, increased reactivity, and hunched back were observed in the treated animals. The LD_{50} was greater than 2,000 mg/kg. A NOEL could not be established for this study. The toxicological significance of this metabolite is uncertain at this time, but it appears to be less toxic than DEF. This study had several deficiencies including only females tested and no analysis of dosing material.

C. SUBCHRONIC TOXICITY

Summary: Seven subchronic studies of variable exposure duration were available for DEF, 3 inhalation studies, 3 oral studies and one dermal study. Only a 13-week inhalation study in rats and a 3-week dermal study in rabbits met the FIFRA guidelines. The clinical signs observed with subchronic exposure to DEF were primarily cholinergic signs. Unlike the acute toxicity studies, ChE inhibition data were available for most of the subchronic studies. In general, DPR does not consider plasma or erythrocyte ChE inhibition in the absence of clinical signs or symptoms an adverse effect because the ChEs in blood have no known physiological function. However, plasma or erythrocyte ChE inhibition are considered indicators of exposure. Only statistically significant brain ChE inhibition was considered an adverse effect. Brain ChE inhibition was one of the more sensitive endpoints with subchronic exposure. A NOEL of 90 ppm (47 mg/kg/day) was observed in mice for brain ChE inhibition (~74% of control activity) which was the only adverse effect reported in the oral subchronic studies for DEF. With inhalation exposure, cholinergic signs, brain ChE inhibition (60% of control activity), impaired retinal function, pale retinal fundus, and fatty droplets in the adrenal cortex were observed in a 13-week rat study with a NOEL of 12.2 mg/m^3 (2.9 mg/kg/day). In the 3-week dermal toxicity study, cholinergic signs, brain ChE inhibition (85% of control activity), acanthosis and hyperkeratosis at the dosing site were observed with a NOEL of 2 mg/kg/day. Other subchronic effects are described under the Reproductive and Developmental Toxicity sections and are summarized under Subchronic Toxicity in the Hazard Identification section.

Inhalation-Rat

Ten Wistar-II rats/sex/group were exposed (nose only) to DEF (95%) at analytical air concentrations of 0, 2, 7 or 32 mg/m^3 (0, 0.5, 1.7 or 7.7 mg/kg/day)[2] for 6 hour/day, 5 days/week for 3 weeks (Thyssen, 1978b). Animals exposed to 32 mg/m^3 exhibited slight behavioral abnormalities, including lethargy and decreased preening. The high-dose animals also had increased absolute and relative adrenal gland and spleen (females only) weights and slight inflammatory lung alterations at necropsy. The increased organ weights were not considered toxicologically significant since there were no apparent treatment-related histological changes in the adrenal gland and spleen. There was a significant reduction in the mean plasma ChE activity at 7 mg/m^3 (M:64%; F:52% of controls) and 32 mg/m^3 (M:36%; F:15% of controls) at study termination. The mean erythrocyte ChE activity was reduced at 32 mg/m^3 (M:76%; F:73% of controls). The mean brain ChE activity was also reduced at 32 mg/m^3 (F:73% of controls). No effects on body weights, hematology, clinical chemistry, urinalyses or gross pathology were reported. The NOEL was 7 mg/m^3 (1.7 mg/kg/day) based on the brain ChE inhibition, clinical signs, and histological changes in the lung. This study had major deficiencies including

[2] Dose was estimated from air concentration in mg/m^3 using Equation 1 in Appendix A. The respiratory rate for a rat was assumed to be 0.24 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979).

C. SUBCHRONIC TOXICITY (cont.)

inadequate exposure duration (< 90 days), inadequate hematology, clinical chemistry, and histopathology, and no analyses of airflow, particle size or temperature in the chambers during exposure.

Inhalation-Rat

Ten Bor: WISW (SPF-Cpb) rats/sex/dose were exposed (nose only) to DEF (98%) at analytical air concentrations of 0, 0.27, 2.6, 13.3 or 62.5 mg/m^3 (0, 0.06, 0.6, 3.2 or 15 mg/kg/day)[2] for 6 hrs/day, 5 days/wk in a two-week range-finding study (Pauluhn, 1991). Particle size analysis indicated that greater than 99% of the particles were less than 3 µm. Therefore, DPR assumed that 100% of the DEF in inhaled air reached the alveoli and was absorbed. At 62.5 mg/m^3, rats exhibited hypoactivity, aggressive behavior, vocalization, piloerection, exophthalmos, bradypnea, dyspnea, and slight hypothermia. At 2.6 mg/m^3 and higher, some females displayed a more pronounced tail-pinch response on day 7. The toxicological significance of this effect is unknown. The mean plasma ChE activity was reduced at 62.5 mg/m^3 (M:42%; F:13% of controls) at the study termination. The mean erythrocyte ChE activity was reduced at 13.3 mg/m^3 (M:62% of controls) and 62.5 mg/m^3 (M:27%; F:25% of controls). Significantly reduced mean brain ChE activity (61% of control activity) was seen in females at 62.5 mg/m^3. In males, a significant reduction in relative liver weights was observed at 13.3 and 62.5 mg/m^3. In females, a significant reduction in absolute spleen weights was observed at 62.5 mg/m^3. The reductions in organ weights were not considered toxicologically significant since there were no treatment-related histological changes in these organs. The acute NOEL was 13.3 mg/m^3 (3.2 mg/kg) based on reduced activity, bradypnea, and vocalization by day 3. The subchronic NOEL was 13.3 mg/m^3 (3.2 mg/kg) based on the clinical signs and brain ChE inhibition. This study was designed only to be a range-finding study; therefore, it did not meet FIFRA guidelines for a subchronic study because the exposure period was short and there was no hematology, clinical chemistry or histopathology examination.

Inhalation-Rat

In a 13-week subchronic inhalation toxicity study, 10 Bor:WISW (SPF-Cpb) rats/sex/dose were exposed (nose only) to analytical air concentrations of DEF at 0, 0.9, 2.4, 12.2 or 59.5 mg/m^3 (0, 0.2, 0.6, 2.9 or 14.3 mg/kg/day)[2] for 6 hours/day, 5 days/week (Pauluhn, 1992). The particle size analysis indicated that greater than 99% of the particles were less than 3µm. Therefore, DPR assumed that 100% of the DEF in inhaled air reached the alveoli and was absorbed. Various clinical signs including reduced motility, bradypnea, dyspnea, increased aggressiveness, miosis, exophthalmos, vocalization, piloerection, convulsions, blepharospasm (spasm in the eyelid muscle resulting in more or less complete closure of the eyelid), and hypothermia (females only) were observed in animals at 59.5 mg/m^3. Some of these signs (reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing and increased startle response) were observed within the first three days of exposure and , therefore, were considered acute effects. Most of these signs do not appear to be cholinergic in origin, but may reflect a localized response in the lungs. However, they appear to be treatment-related since none of these were observed in the lower treatment groups or the controls. There was no treatment-related effect on reflexes, body weight or clinical chemistry. The mean erythrocyte count, hematocrit, and hemoglobin values were reduced significantly in males at 59.5 mg/m^3 (8%, 7%, and 8%, respectively). The mean erythrocyte count, hematocrit and hemoglobin values were also reduced in females (3%, 7%, and 6%, respectively), but these differences were not statistically significant. At study termination, the mean plasma ChE activity

C. SUBCHRONIC TOXICITY (cont.)

was reduced at 12.2 mg/m^3 (F:60% of controls) and 59.5 mg/m^3 (M:51%; F:33% of controls). The mean erythrocyte ChE activity was also reduced at 12.2 mg/m^3 (M:35%; F:36% of controls) and 59.5 mg/m^3 (M:19%; F:13% of controls). The mean brain ChE activity was significantly reduced at 59.5 mg/m^3 only (M&F:60% of controls). Pale or mottled retinal fundus were noted in females at 59.5 mg/m^3 with the ophthalmological examination. Animals at 59.5 mg/m^3 had evidence of impaired retinal function based on reduced a and b waves in the electroretinographic (ERG) examination; however, histological examination of the eye revealed no evidence of retinal degeneration. Fine fatty droplets in the adrenal cortex and elevated absolute and relative adrenal gland weights were also seen in rats at 59.5 mg/m^3. The NOEL was 12.2 mg/m^3 (2.9 mg/kg/day) based on the clinical signs, brain ChE inhibition, impaired retinal function, pale retinal fundus, fatty droplets in the adrenal gland, and increased adrenal weights. This study was found acceptable by DPR based on FIFRA guidelines.

Diet-Mouse

In a pilot study, 15 CD-1 mice/sex/group were fed DEF (97.7%) in the diet at 0, 10, 30, 90 or 270 ppm (M: 0, 3.4, 9.4, 40 or 140 mg/kg/day; F: 0, 5.6, 14.3, 54 or 132 mg/kg/day) for 8 weeks (Hayes, 1985). No clinical signs or deaths were observed. The mean food consumption was higher in the males at 90 ppm (33%) and 270 ppm (51%) and in females at 90 ppm (29%). There was no effect on body weight gain. The mean plasma ChE activity was reduced at 10 ppm (M:36%; F:29% of controls), 30 ppm (M:13%; F:8% of controls), 90 ppm (M:7%; F:4% of controls), and 270 ppm (M:5%; F:4% of controls). The mean erythrocyte ChE activity was reduced at 30 ppm (M:63%; F:56% of controls), 90 ppm (M&F:36%), and 270 ppm (M:27%; F:31% of controls). The mean brain ChE activity was only reduced at 270 ppm (M:74%; F:71% of control activity). The NOEL was 90 ppm (M: 40 mg/kg/day; F: 54 mg/kg/day) based on the brain ChE inhibition. This study was designed as a pilot study for an oncogenicity study and, therefore, the exposure period was short. In addition, there was no histopathological examination, no clinical chemistry or hematology, and no analysis of the diet.

Diet-Rat

Groups of male and female Sprague-Dawley rats were fed diets containing DEF at 0, 5, 10, 20, 50 or 100 ppm (0, 0.25, 0.5, 1.0, 2.5 or 5.0 mg/kg/day)[3] for 3 months (Root and Doull, 1966). A NOEL of 5 ppm (0.25 mg/kg/day) was reported, but the toxic effects were not indicated. This study had major deficiencies including no summary of the incidence of clinical signs, body weights, food consumption, pathology findings and no clinical chemistry or hematology.

Diet-Dog

DEF was also administered to groups of male and female beagle dogs in the feed at 0, 5, 10, 20, 50 or 100 ppm (0, 0.125, 0.25, 0.5, 1.25 or 2.5 mg/kg/day)[4] for 3 months (Root and Doull, 1966). Again, a NOEL of 5 ppm (0.125 mg/kg/day) was reported, but the toxic effects were not indicated. This study also had major deficiencies including no summary of the

[3] Estimated assuming that for a rat 1 ppm in the diet is equivalent to 0.05 mg/kg/day (FDA, 1959).

[4] Estimated assuming that for a dog 1 ppm in the diet is equivalent to 0.025 mg/kg/day (FDA, 1959).

C. SUBCHRONIC TOXICITY (cont.)

incidence of clinical signs, body weights, food consumption, pathology findings and no clinical chemistry or hematology.

Dermal-Rabbit

A subchronic dermal toxicity study was conducted in which DEF (99%) was applied topically to the shaved backs of 5 New Zealand white rabbits/sex/dose at 0, 2, 11 or 29 mg/kg/day (actual) for 6 hrs/day, 5 days/wk for 3 weeks (Sheets *et al.*, 1991). An additional 5 rabbits/sex were added to the control and high-dose group for a recovery study. Animals in the recovery groups were held for another 2 weeks after the last exposure. Clinical signs (muscle fasciculations, dried, cracked or flaking skin, erythema, tremors, decreased motor activity, anal stain, red conjunctiva, clear lacrimation, clear nasal discharge, edema, urine stain, and increased reactivity) were seen in both sexes at either 11 or 29 mg/kg/day (Table 4). Red conjunctiva, lacrimation, and anal stains were also seen in a few animals at 2 mg/kg/day. The investigators attributed the red conjunctiva and lacrimation to the plastic collars the rabbits wore during exposure to prevent licking of the application site. To support this conclusion, they noted that these signs resolved within one day in all of the recovery groups after the collars were removed. The investigators considered the incidence of anal stains to be treatment-related; however, the toxicological significance of this sign at 2 and 11 mg/kg/day is uncertain because the incidence was similar to the control group. A reduction in mean body weights (M:15%; F13%) and food consumption (M:30%; F:29%) was seen in both sexes at 29 mg/kg/day by study termination. No compound-related effects were observed with the ophthalmological and gross pathological examinations. Clinical pathological findings included an increased number of segmented white blood cells, a decreased number of lymphocytes, and an increase in blood urea nitrogen (BUN) levels in animals at 29 mg/kg/day. The toxicological significance of the changes in hematological and clinical chemistry values is uncertain without accompanying histological changes. The mean plasma ChE activity was reduced at 2 mg/kg/day (M:82%; F:89% of controls), 11 mg/kg/day (M:43%; F:46% of controls), and 29 mg/kg/day (M:27%; F:26% of controls). A reduction in the mean erythrocyte ChE activity was also observed at 2 mg/kg/day (M:89%; F:80% of controls), 11 mg/kg/day (M&F:30% of controls), and 29 mg/kg/day (M:28%; F:20% of controls). The mean brain ChE activity was significantly reduced at 11 mg/kg/day (M:86%; F:85% of controls) and 29 mg/kg/day (M:68%; F:62% of controls). Microscopic findings were limited to acanthosis and hyperkeratosis, which were observed in the skin at the dosing site of both sexes at 11 and 29 mg/kg/day. The hyperkeratosis was of minimal severity at 2 mg/kg/day and apparently reversible based on the decreased incidence in the high-dose recovery group. Therefore, they were not considered toxicologically significant. Based on the muscle fasciculations, brain ChE inhibition (85-86% of controls), and microscopic lesions in the skin seen at 11 mg/kg/day, the subchronic NOEL for this study was 2 mg/kg/day. An acute NOEL was estimated to be 11 mg/kg/day for this study based on the onset of muscle fasciculations in 9 of 10 animals at 29 mg/kg/day on day 2. DPR found this study acceptable based on the FIFRA guidelines.

D. CHRONIC TOXICITY/ONCOGENICITY

Summary: Four chronic toxicity studies were available, including a 90-week mouse study, two 2-year rat studies, and a 1-year dog study. All four studies administered DEF to the animals in the diet. All of the studies met FIFRA guidelines, except one of the rat studies. Mice, rats, and dogs exposed to DEF orally for one year or longer all had evidence of marked

Table 4. Incidence of Mortalities, Clinical Signs and Microscopic Lesions in Rabbits with Dermal Exposure to DEF for 3 Weeks

	Dose Level (mg/kg/day)							
	0		2		11		29	
	M	F	M	F	M	F	M	F
Death	0[a]	0	0	0	0	0	1(18)	4(12)
Clinical Signs								
Red conjunctiva	2(5)	9(3)	2(7)	2(3)	2(3)	3(2)	3(3)	5(2)
Muscle Fasciculations	0	0	0	0	2(8)	4(5)	10(2)	10(1)
Dried, cracked or flaking skin	0	0	0	0	3(17)	2(14)	10(10)	10(11)
Tremors	0	0	0	0	0	0	9(6)	10(5)
Hypoactivity	0	0	0	0	0	0	7(4)	10(5)
Anal stain	0	1(16)	2(8)	0	2(8)	1(12)	9(13)	1(10)
Erythema	0	0	0	0	2(13)	1(18)	3(6)	7(7)
Clear lacrimation	2(3)	2(3)	1(1)	0	0	1(6)	4(2)	3(3)
Clear nasal discharge	0	0	0	0	0	0	1(10)	4(6)
Edema	0	0	0	0	0	0	2(5)	0
Urine stain	0	0	0	0	0	0	1(13)	1(11)
Increased reactivity	0	0	0	0	1(4)	0	0	1(5)
Salivation	0	0	0	0	0	0	0	1(13)
Microscopic Lesions								
Acanthosis	0	0	0	0	4	1	5	2
Hyperkeratosis	0	0	1	1	5	4	8	7

[a] Five animals/sex/dose, except for the control and high-dose group which had 10 animals/sex/dose, half of which were used for a recovery study. Day of onset for each clinical sign indicated in parentheses next to incidence for each group.

anemia based on reduced hematological values. Significant brain ChE inhibition was observed in all three species. Gastrointestinal effects were seen in both mice and rats including vacuolar degeneration and hyperplasia of the small intestine. Degeneration or pigmentation of the adrenal glands were also seen in both mice and rats. Liver effects were also noted in both species (hypertrophy in mice and cytoplasmic vacuolation in rats). Other treatment-related histopathological changes were only seen in one species. Extramedullary hematopoiesis was observed in the spleen of mice. Ocular lesions were also seen in rats, including cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis/uveitis, bilateral unrecordable ERG

responses, bilateral retinal atrophy, and optical nerve atrophy. There was no evidence of oncogenicity in rats; however, in mice there was a significant increase in adenocarcinomas of the small intestine in both sexes, liver hemangiosarcomas in males, and alveolar/bronchiolar adenomas in females. Not only was there an increase in tumors at more than one site, but there was a significant increase in one tumor type (adenocarcinomas of the small intestine) in both sexes by trend analysis ($p < 0.01$). The lowest NOEL was 4 ppm (0.2 mg/kg/day) in rats based on hyperplasia and vacuolar degeneration of the small intestine and anemia.

Diet-Mouse

In a 90-week study, 50 CD-1 mice/sex/group were fed DEF (98.6% purity) in the diet at 0, 10, 50 or 250 ppm (M: 0, 1.5, 8.4 or 48.1 mg/kg/day; F:0, 2.0, 11.3 or 63.1 mg/kg/day) (Hayes, 1989). The survival rate was significantly reduced in both sexes at 250 ppm (M:50%; F:38%). The early deaths occurred primarily in the last five months, although there was a significant increase in deaths during months 12 to 18. Enlarged abdomens were seen in both sexes at 250 ppm during weeks 14 to 26. Paleness, loose stools and perineal staining were common in the 250 ppm animals in the second year and coincided with the period of increased mortality. The loose stools and perineal staining were not attributed to cholinesterase inhibition due to the late onset of these effects. Body weights increased for both sexes at 250 ppm after week 13. At necropsy, a significant increase in fluid-filled or dilated intestines and cecum was observed macroscopically in the 250 ppm animals. The males at 50 and 250 ppm had a significant increase in the incidence of an enlarged spleen. A significant increase in the absolute weights of the liver, spleen (males only) and heart (males only) was found in the 250 ppm animals. At study termination, the mean plasma ChE activity was significantly reduced at 10 ppm (M:33%; F:35% of controls), 50 ppm (M:9%; F:7% of controls), and 250 ppm (M:6%; F:3% of controls). The mean erythrocyte ChE activity was also reduced at 10 ppm (M&F:82% of controls), 50 ppm (M:58%; F:63% of controls), and 250 ppm (M:45%; F:50% of controls). There was a statistically significant reduction in the mean brain ChE activity at 10 ppm (M:91% of controls), 50 ppm (M:87% of controls), and 250 ppm (M:62%; F:73% of controls). Both sexes at 250 ppm had evidence of anemia based on significant reductions in their mean erythrocyte counts (M:29%; F:13%), hemoglobin (M:18%; F:13%) and hematocrits (M:20%;F:11%) values and increases in their mean corpuscular volumes (M:16%) and mean corpuscular hemoglobin (M:20%) values at study termination. Females at 50 ppm also had significant reductions in their mean erythrocyte counts (10%), hemoglobin (8%), and hematocrits (8%) at study termination.

A significant increase in numerous non-neoplastic lesions in the intestines, liver, adrenal gland, and spleen were observed microscopically in animals at 250 ppm (Tables 5 and 6). The incidence of mucosal hyperplasia in the small intestine, dilated/edematous cecum or small intestine, necrosis/ulceration of the rectum, and adrenal degeneration/ pigmentation were significant at 250 ppm with dose-related trends. The incidences of vacuolar degeneration in the small intestine and extramedullary hematopoiesis in the spleen (males only) were significant at 50 and 250 ppm with dose-related trends. The incidence of focal atypia (group of abnormal appearing cells) in the small intestine exhibited a dose-related trend, although the increase was not significant when compared with the concurrent controls. The study pathologist considered the focal atypia pre-neoplastic, although he made no comment about the mucosal hyperplasia which could also be considered pre-neoplastic. The study pathologist attributed the vacuolar degeneration to the inability of the epithelial cells to "absorb or secrete products", resulting in fluid accumulation. The extramedullary hematopoiesis in the spleen may be related to the

Table 5. Incidence of Non-neoplastic Microscopic Lesions in Male Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Vacuolar degeneration	0/50^{+++}	1/50	8/50**	28/50***
	(0%)	(2%)	(16%)	(56%)
Mucosal hyperplasia	0/50^{+++}	0/50	1/50	22/50***
	(0%)	(0%)	(2%)	(44%)
Focal atypia	0/50^{+++}	0/50	0/50	4/50
	(0%)	(0%)	(0%)	(8%)
Dilated/distended	0/50^{+++}	0/50	2/50	7/50**
	(0%)	(0%)	(4%)	(14%)
Cecum				
Dilated/edematous	4/50^{++}	8/50	6/50	13/50*
	(8%)	(16%)	(12%)	(26%)
Rectum				
Necrosis/ulceration	0/45^{+++}	1/49	1/47	10/46***
	(0%)	(2%)	(2%)	(22%)
Liver				
Hypertrophy	1/50^{++}	0/50	1/50	4/50
	(2%)	(0%)	(2%)	(8%)
Adrenal				
Degeneration/pigment.	17/50^{+++}	15/50	21/50	39/50***
	(34%)	(30%)	(42%)	(78%)
Spleen				
Hematopoiesis	6/50^{+++}	6/50	14/50*	19/50**
	(12%)	(12%)	(28%)	(38%)

[a] The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.

$^{++}$, $^{+++}$ A significant trend based on a dose-weighted chi-square test at $p < 0.01$, and 0.001, respectively (Peto *et al.*, 1980).

*, **, *** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

Table 6. Incidence of Non-neoplastic Microscopic Lesions in Female Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Vacuolar degeneration	0/50^{+++}	0/50	11/50***	28/50***
	(0%)	(0%)	(22%)	(56%)
Mucosal hyperplasia	1/50^{+++}	0/50	0/50	19/50***
	(2%)	(0%)	(0%)	(38%)
Focal atypia	0/50^{+}	0/50	0/50	1/50
	(0%)	(0%)	(0%)	(2%)
Dilated/distended	2/50^{+++}	0/50	1/50	18/50***
	(4%)	(0%)	(2%)	(36%)
Cecum				
Dilated/edematous	7/50^{+++}	3/50	4/50	20/50**
	(14%)	(6%)	(8%)	(40%)
Rectum				
Necrosis/ulceration	2/50^{+++}	0/46	1/50	14/49**
	(4%)	(0%)	(2%)	(29%)
Liver				
Hypertrophy	0/50^{+++}	2/50	0/50	6/50*
	(0%)	(4%)	(0%)	(12%)
Adrenal				
Degeneration/pigment.	18/50^{+++}	26/50	22/50	38/49***
	(36%)	(52%)	(44%)	(78%)
Spleen				
Hematopoiesis	16/50	14/50	18/50	20/50
	(32%)	(28%)	(36%)	(40%)

[a] The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.

$^{+}$, $^{+++}$ A significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*, **, *** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

D. CHRONIC TOXICITY/ONCOGENICITY (cont.)

anemia and enlarged spleen, but these findings were not usually present in the same animal at the same time. The increase in adrenal degeneration/pigmentation was considered by the study pathologist to be an enhancement of a common age-related lesion that may be due to stress. The incidence of liver hypertrophy exhibited a dose-related trend in both sexes, but was only significant in females at 250 ppm by pairwise statistical comparison to the concurrent controls. There was no correlation of the liver hypertrophy observed histologically with the increased liver weights.

A significant increase in several neoplastic lesions was reported in mice fed DEF in the diet at the high dose (Tables 7 and 8) (Hayes, 1989). There was a significant increase in liver hemangiosarcomas in males at 250 ppm that exhibited a dose-related trend. Among the animals with liver hemangiosarcomas most also had hemorrhage and/or necrosis in the liver (M: 0/1, 1/1, 4/4, 6/7; F: 2/2, ½, 2/2, 1/1). The incidence of liver hemangiosarcomas in the males at 250 ppm was outside the historical control range for males reported by this laboratory (0-6%). However, the historical control data consisted of only 50 mice/sex/study from three studies.

There was an increase in adenocarcinomas of the small intestine in both sexes which were significant by trend analysis primarily due to the response at the high dose. The increase in these tumors was very highly significant by pairwise comparison with concurrent controls in males ($p < 0.001$), but not in females at 250 ppm ($p = 0.054$). Some of these tumors were associated with inflammatory responses. The reported historical control range for this laboratory was 0% for both sexes. In addition, mice with adenocarcinomas often had focal atypia, mucosal hyperplasia, and/or vacuolar degeneration of the small intestines, too. The investigators suggested that these lesions in the small intestine are interrelated based on their multiplicity and dose relationship.

The incidence of alveolar/bronchiolar adenomas was also significantly higher in females at 250 ppm and was significant by trend analysis essentially due to the response at the high dose. The incidence at the high dose was outside the laboratory's historical control range for these tumors in females (0-14%). There was also a significant positive trend in other potentially pre-neoplastic lesions in the lungs of females including epithelialization (0/50, 1/50, 4/50, 5/50) and focal hyperplasia (3/50, 4/50, 3/50, 8/50). The increase in epithelialization was significant at 250 ppm. The multiplicity of the lung lesions (focal hyperplasia and alveolar/bronchiolar adenomas and carcinomas) was elevated in the females at 250 ppm (0/50, 0/50, 1/50, 9/50).

Small intestine adenocarcinomas and liver hemangiosarcomas were present in several males at 250 ppm that died during the study (M - 1/16, 0/14, 3/21, 12/30; F - 1/19, 1/17, 2/22, 2/31) and may account for some of the early deaths. There was no association with the early deaths and the tumor incidence in females. The liver hemangiosarcomas, small intestine adenocarcinomas and alveolar/bronchiolar adenomas were first seen in females on week 46 (50 ppm), 69 (250 ppm), and 74 (250ppm), respectively.

The NOEL was 10 ppm (M:1.5 mg/kg/day; F: 2.0 mg/kg/day) based on vacuolar degeneration of the small intestine, spleen hematopoiesis, hematological changes, and reduced brain ChE activity (87% of controls) at 50 ppm. Although a statistically significant reduction in the mean brain ChE activity was observed in males at 10 ppm (91% of controls), the reduction was not considered toxicologically significant for several reasons. First, the reduction at 10 ppm was less than 10% of control activity. Second, no cholinergic signs were observed at either 10

D. CHRONIC TOXICITY/ONCOGENICITY (cont.)

Table 7. Incidence of Neoplastic Microscopic Lesions in Male Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Adenocarcinoma[b]	0/47[+++]	0/48	0/47	9/46***
	(0%)	(0%)	(0%)	(20%)
Liver				
Hemangiosarcoma[c]	1/47[++]	1/48	4/47	7/46*
	(2%)	(2%)	(9%)	(15%)
Lungs				
Alveolar/bronchiolar	11/47	9/48	5/47	9/46
adenoma[d]	(23%)	(19%)	(11%)	(20%)
Alveolar/bronchiolar	3/47	5/48	4/47	3/46
carcinoma[e]	(6%)	(10%)	(9%)	(7%)
Alveolar/bronchiolar	11/47	13/48	9/47	11/46
tumors - combined	(23%)	(27%)	(19%)	(24%)

[a] The denominator is the number of animals at risk (excluding those that died before the first tumor was observed or 52 weeks, whichever came first); the number in parentheses represents the incidence in percentage.
[b] First small intestine adenocarcinoma observed on week 75 at 250 ppm.
[c] First liver hemangiosarcoma observed on week 59 at 50 ppm.
[d] First alveolar/bronchiolar adenoma observed on week 57 at 10 ppm.
[e] First alveolar/bronchiolar carcinoma observed on week 57 at 10 ppm.
++, +++ A significant trend based on a dose-weighted chi-square test at $p < 0.01$ and 0.001, respectively.
*,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$ and 0.001, respectively.

or 50 ppm. Third, only mild cholinergic signs (loose stools and perineal staining) were observed at 250 ppm despite a reduction in the mean brain ChE activities of 63% and 73% of control activity in males and females, respectively. DPR found this study acceptable based on the FIFRA guidelines.

Diet-Rat

Groups of 24 Sprague-Dawley rats/sex/group were fed DEF (97.7%) in the diet at 0, 5, 25, 100 or 250 ppm (0, 0.25, 1.25, 5.0 or 12.5 mg/kg/day)[5] for 2 years (Root *et al.*, 1967).

[5] Estimated assuming that for a rat 1 ppm in the diet is equivalent to 0.05 mg/kg/day (FDA, 1959).

D. CHRONIC TOXICITY/ONCOGENICITY (cont.)

Table 8. Incidence of Neoplastic Microscopic Lesions in Female Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Adenocarcinoma[b]	0/49[++]	1/45	0/44	4/47[c]
	(0%)	(2%)	(0%)	(9%)
Liver				
Hemangiosarcoma[d]	2/49	2/47	2/47	1/48
	(4%)	(4%)	(4%)	(2%)
Lungs				
Alveolar/bronchiolar	5/49[+++]	5/45	2/44	15/47**
adenoma[e]	(10%)	(11%)	(5%)	(32%)
Alveolar/bronchiolar	1/49	2/45	0/44	2/47
carcinoma[f]	(2%)	(4%)	(0%)	(4%)
Alveolar/bronchiolar	6/49[+++]	7/45	2/44	16/47**
tumors - combined	(12%)	(16%)	(5%)	(34%)

[a] The denominator is the number of animals at risk (excluding those that died before the first tumor was observed or 52 weeks, whichever came first); the number in parentheses represents the incidence in percentage.
[b] First small intestine adenocarcinoma observed on week 69 at 250 ppm.
[c] Not significantly different (p = 0.054) from the control group based on Fisher's exact test.
[d] First liver hemangiosarcoma observed on week 46 at 50 ppm.
[e] First alveolar/bronchiolar adenoma observed on week 74 at 250 ppm.
[f] First alveolar/bronchiolar carcinoma observed on week 75 at 250 ppm.
++, +++ A significant trend based on a dose-weighted chi-square test at p < 0.01 and 0.001, respectively.
** Significantly different from the control group based on the Fisher's exact test at p < 0.01.

There was no effect on survival even at the highest dose level. The mean body weight gain in males at 100 ppm was reduced (~12% relative to controls) by the end of the study. The females fed DEF at 250 ppm also had significantly reduced (~20% relative to controls) mean body weight gain. The mean plasma ChE activity was significantly reduced at 25 ppm (M:69%; F:65% of controls), 100 ppm (M:31%; F:26% of controls), and 250 ppm (M:22%; F:18% of controls). A reduction in the mean erythrocyte ChE activity was seen at 25 ppm (M:53%; F:42% of controls), 100 ppm (M:27%; F:18% of controls), and 250 ppm (M:15%; F:12% of controls). The mean brain ChE activity was significantly reduced at 100 ppm (F:69% of controls) and 250 ppm (M:57%; F:32% of controls). An increased incidence of liver cytoplasmic vacuolation was found in females at the 100 and 250 ppm dose levels. The NOEL was 25 ppm (1.25 mg/kg/day) based on the liver cytoplasmic vacuolation, reduced weight gain and brain ChE inhibition (F:69% of controls). This study had major deficiencies including incomplete histopathological

examination, no hematology or clinical chemistry data, no analysis of dosing material, no individual data, and intercurrent disease.

Diet-Rat

A combined chronic toxicity/oncogenicity/neurotoxicity study was conducted in which Fischer 344 rats were fed DEF (98.5%) in the diet at 0, 4, 40 or 320 ppm (M: 0, 0.2, 1.8 or 16.8 mg/kg/day; F: 0, 0.2, 2.3 or 21.1 mg/kg/day) for 2 years (Christenson, 1992). Fifty rats/sex/dose were assigned to the oncogenicity study, 20 rats/sex were assigned to the control and 320 ppm groups as interim sacrifice animals for the chronic toxicity study, and 20 rats/sex/dose were assigned to the neurotoxicity study. The incidences of a number of clinical signs were higher in the 320 ppm rats, including pale eyes, ocular opacity, rough coats, rash, raised zones of the skin, urine stains, clear discharge (origin not reported), soft feces, and diarrhea. The mean body weight gains were reduced in both sexes at 320 ppm (~15%) at study termination. Body temperature reductions occurred more frequently in the 40 and 320 ppm rats, although not in a consistent or dose-related manner.

Significant decreases in several hematological values (erythrocyte counts, hemoglobin, and hematocrits) were found in blood drawn from the 40 and 320 ppm rats at 6 and 12 months, but by 18 and 24 months some of these values had returned to normal levels. In fact, these values had actually increased in the 320 ppm rats when compared to controls, possibly from some compensatory mechanism(s). These hematological changes were considered an adverse effect based on the evidence in hens that DEF is hydrolyzed in the gut to nBM which can cause methemoglobinemia and eventual cell lysis (Abou-Donia, 1979: Abou-Donia *et al.*, 1979a&b). The NOEL for the hematological changes was 4 ppm (M & F: 0.2 mg/kg/day). Several clinical chemistry values, including a decrease in plasma glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total protein, albumin, and globulin and an increase in BUN, triglycerides, and creatine kinase (CK), were also significantly different in the 40 and 320 ppm groups compared to controls at 6 months. A few of these values had also returned to control levels at both dose levels by study termination, including AST, ALT, CK, and triglycerides. Other values had only returned to control levels in the 40 ppm group (total protein, albumin, globulin, and BUN). The toxicological significance of these changes in clinical chemistry values is uncertain, especially in the absence of any histological changes in the liver, kidney or heart. There was a reduction in the mean plasma ChE activity at 4 ppm (M:84%; F:94% of controls), 40 ppm (M:44%; F:40% of controls), and 320 ppm (M:20%; F:17% of controls) at study termination. The mean erythrocyte ChE activity was reduced at 40 ppm (M:73%; F:72% of controls) and 320 ppm (M:52%; F:53% of controls) while the mean brain ChE activity was only reduced in the 320 ppm rats (M:40%; F:32% of controls). The NOEL for brain ChE inhibition was 40 ppm (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day).

Ophthalmologic examination revealed an increased incidence of cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis and/or uveitis in both sexes at 320 ppm at study termination (Tables 9 and 10). These effects were not seen in the 1-year interim sacrifice animals. An increased incidence of bilateral unrecordable (flat) ERG responses was seen in 2-year-old rats of both sexes at 320 ppm. Microscopic examination of the eye also revealed bilateral retinal atrophy (1- and 2-year) and optical nerve atrophy (2-year) in both sexes at 320 ppm. Because the cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis/uveitis, and optical nerve atrophy were not seen in the one-year rats, the study pathologist

Table 9. Incidence of Ophthalmologic and Microscopic Lesions in Male Rats Fed DEF for 2 Years

	Dose Level (ppm)			
	0	4	40	320
Ophthalmology Examination				
Posterior, subcapsular	5/36^{+++}	4/30	5/36	27/32***
or complete cataract	(14%)	(13%)	(14%)	(84%)
Lens Opacity	6/36	4/30	3/36	8/32
	(17%)	(13%)	(8%)	(25%)
Diffuse or focal	21/36^{+++}	20/30	26/36	31/32***
corneal opacity	(58%)	(67%)	(72%)	(97%)
Corneal neovascularization	2/36^{+++}	6/30	1/36	15/32***
	(5%)	(20%)	(3%)	(47%)
Iritis and/or uveitis	3/36^{+++}	5/30	7/36	31/32***
	(8%)	(17%)	(19%)	(97%)
Electroretinographic Examination				
Bilateral unrecordable responses	0/15^{+++}	2/9	0/15	11/13***
	(0%)	(22%)	(0%)	(85%)
Microscopic Examination				
Bilateral retinal atrophy	1/50^{+++}	0/50	0/50	50/50***
	(2%)	(0%)	(0%)	(100%)
Optic nerve atrophy	10/50^{+++}	6/50	6/50	32/50***
	(20%)	(12%)	(12%)	(64%)
Small intestine				
Vacuolar degeneration	0/50^{+++}	1/50	24/50***	37/50***
	(0%)	(2%)	(48%)	(74%)
Hyperplasia	0/50^{+++}	3/50	23/50***	34/50***
	(0%)	(6%)	(46%)	(68%)
Adrenal vacuolar degeneration	6/50^{+++}	6/49	9/50	35/49***
	(12%)	(12%)	(18%)	(71%)

$^{+}$,$^{+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$ and 0.001, respectively.

D. CHRONIC TOXICITY/ONCOGENICITY (cont.)

Table 10. Incidence of Ophthalmologic and Microscopic Lesions in Female Rats Fed DEF for 2 Years

	Dose Level (ppm)			
	0	4	40	320
Ophthalmology Examination				
Posterior, subcapsular	4/41^{+++}	6/38	6/34	15/32***
or complete cataract	(10%)	(16%)	(18%)	(47%)
Lens Opacity	9/41^{+++}	8/38	5/34	20/32***
	(22%)	(21%)	(15%)	(62%)
Diffuse or focal	20/41^{+++}	27/38*	20/34	31/32***
corneal opacity	(49%)	(71%)	(59%)	(97%)
Corneal neovascularization	11/41^{+++}	7/38	4/34	19/32**
	(27%)	(18%)	(12%)	(59%)
Iritis and/or uveitis	3/41^{+++}	5/38	5/34	29/32***
	(7%)	(13%)	(15%)	(91%)
Electroretinographic Examination				
Bilateral unrecordable responses	1/16^{+++}	2/16	0/13	7/8***
	(6%)	(12%)	(0%)	(88%)
Microscopic Examination				
Bilateral retinal atrophy	0/50^{+++}	2/50	0/50	40/50***
	(0%)	(4%)	(0%)	(80%)
Optic nerve atrophy	15/50^{+++}	12/50	12/50	34/50***
	(30%)	(24%)	(24%)	(68%)
Small intestine				
Vacuolar degeneration	0/50^{+++}	0/50	19/50***	35/50***
	(0%)	(0%)	(38%)	(70%)
Hyperplasia	1/50^{+++}	0/50	11/50**	30/50***
	(2%)	(0%)	(22%)	(60%)
Adrenal vacuolar degeneration	10/50^{+++}	6/50	16/50	41/50***
	(20%)	(12%)	(32%)	(82%)

$^{+,+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*,**,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

concluded that these effects were secondary to the retinal atrophy. The NOEL for ocular lesions was 40 ppm (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day).

No dose-related increases were found in the microscopic lesions of the brain, spinal cord and sciatic nerve of rats assigned to the neurotoxicity study. Some studies suggest that rodents (especially Fischer 344 rats) are less sensitive to OPIDN (Abou-Donia, 1981; Somkuti *et al.*, 1988; De Bleeker *et al.*, 1992). The susceptibility of rodents to OPIDN appears to be variable based on studies by other investigators (Padilla and Veronesi, 1988; Veronesi *et al.*, 1991; Moretto *et al.*, 1992; Inui *et al.*, 1993). Differences in age, regeneration of peripheral nerves, aging and resynthesis of NTE, and metabolism have been suggested as possible explanations for the variable response among rodents (Moretto *et al.*, 1992; Veronesi *et al.*, 1991). Since chemicals that produce OPIDN can affect both sensory and motor nerves (Abou-Donia, 1981), it is possible that the degeneration of the retina and optic nerve observed in this study is another, perhaps more sensitive, sign of OPIDN in rats.

There was an increase in other non-ocular lesions in 2-year-old rats of both sexes at 320 ppm, including vacuolar degeneration of the adrenal glands and small intestine and hyperplasia of the small intestine. The incidence of the vacuolar degeneration and hyperplasia of the small intestine was also increased in 2-year-old rats at 40 ppm. An increase incidence of vacuolar degeneration of the small intestine was also seen in rats of both sexes at the 1-year interim sacrifice. The incidence in 1-year-old males was 0/20, 0/10, 7/10, and 18/20 at 0, 4, 40 and 320 ppm, respectively. The incidence in 1-year-old females was 0/20, 0/10, 8/10, and 16/20 at 0, 4, 40, and 320 ppm, respectively. The lesions in the small intestine correlated with the gross findings of thickened and white discoloration. The adrenal lesions correlated with the gross finding of enlargement and increased adrenal weights. The NOELs for the lesions in the small intestine and adrenal glands were 4 ppm (M & F: 0.2 mg/kg/day) and 40 ppm (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day), respectively. A decrease in the incidence of chronic nephropathy was seen in the 2-year-old rats. The incidence among males was 50/50, 50/50, 46/50, and 34/50 at 0, 4, 40 and 320 ppm, respectively. The incidence among females was 39/50, 45/50, 30/50, and 25/50 at 0, 4, 40, and 320 ppm, respectively. There were no dose-related increases in the incidence of benign or malignant tumors. The overall NOEL for this study was 4 ppm (M & F: 0.2 mg/kg/day) based on the hyperplasia and vacuolar degeneration of the small intestine, and hematological changes. This study was acceptable to DPR.

Diet-Dog

In a chronic dog study, 4 beagle dogs/sex/group were administered DEF (98.5%) in the feed at 0, 4, 16 or 64 ppm (M: 0, 0.1, 0.4 or 1.7 mg/kg/day; F: 0, 0,1, 0.4 or 2.0 mg/kg/day) for 1 year (Christenson, 1991). There were no treatment-related differences in body weights, food consumption, clinical signs, clinical chemistry, brain ChE, urinalysis, palpable masses, gross pathologic, histopathologic and ophthalmologic lesions. At study termination, the mean plasma ChE activity was significantly depressed at 16 ppm (M:67% of controls) and 64 ppm (M:38%; F:52% of controls). The mean erythrocyte ChE activity was also reduced (M:87%; F:84% of controls) at 64 ppm. Slight reductions in the mean RBC count (9-14%), hemoglobin value (6-13%), and hematocrit (8-12%) were observed in females at 64 ppm on days 91, 182, 273 and 364. Although the reductions in the means were greatest on day 364, the differences were only statistically significant on day 273. The NOEL was 16 ppm (0.4 mg/kg/day) based on the hematological changes in females. This study was acceptable to DPR based on the FIFRA guidelines.

E. GENOTOXICITY

Summary: Five genotoxicity tests were available for DEF including an Ames assay, an *in vitro* chromosomal aberrations assay with Chinese hamster ovary cells, two *in vitro* sister chromatid exchange assay with Chinese hamster V79 cells, and an unscheduled DNA synthesis assay with rat primary hepatocytes. Three of these tests met FIFRA guidelines (which refer to the Toxic Substances Control Act (TSCA) guidelines for genotoxicity studies). There was no evidence of genotoxicity in any of the five available studies.

Gene Mutation

DEF (98.5%) did not produce an increase in the mutation frequency in a mutagenicity assay using *Salmonella typhimurium* strains, TA98, TA100, TA1535, TA1537 and TA1538 at concentrations ranging from 667 to 10,000 µg/plate with and without metabolic activation (Curren and Gentry, 1989). The assay was acceptable to DPR based on the FIFRA guidelines.

Chromosome Effects

No increase in chromosomal aberrations was seen in Chinese hamster ovary cells exposed to DEF (98.5%) at concentrations of 0.007 to 0.1 µl/ml with metabolic activation and at 0.004 to 0.05 µl/ml without activation (Putman and Morris, 1989). This study was acceptable to DPR.

Other Genotoxic Effects

Chen *et al.* (1982a&b) found no increase in sister chromatid exchanges in Chinese hamster V79 cells exposed to DEF (95.7%) at concentrations from 2.5 to 20 µg/ml with and without metabolic activation. Nicholas and Van Den Berghe (1982) also reported no increase in sister chromatid exchanges in Chinese hamster V79 cells exposed to DEF at concentrations up to 60 µM (18.9 µg/ml) without metabolic activation.

In an unscheduled DNA synthesis assay, no increase in the average grains per nucleus was observed in rat primary hepatocytes exposed to DEF (98.5%) at concentrations between 0.0001 and 0.03 µl/ml (Curren, 1989). DPR found this study acceptable.

F. REPRODUCTIVE TOXICITY

Summary: Only one reproductive toxicity study was available for DEF. The test compound was administered to rats by the oral route. The study met FIFRA guidelines. Several reproductive effects were seen in this study. The reproductive effects included reductions in the fertility, birth, and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, and gross pathological lesions in pups. The reproductive NOEL was 32 ppm (2.4 mg/kg/day). Other non-reproductive effects that were observed included brain ChE inhibition and reduced body weight gains. The parental NOEL was 4 ppm (0.3 mg/kg/day) based on the brain ChE inhibition. Based on a cross-fostering study, the reproductive effects in the pups, such as reduced birth and viability indices, reduced neonatal pup weights, clinical signs and gross pathological lesions were probably related to maternal toxicity rather than a direct effect of DEF on the pups.

F. REPRODUCTIVE TOXICITY (cont.)

Diet-Rat

In a two-generation rat reproduction study, 30 Sprague Dawley rats/sex/group/ generation were fed DEF (98.5%) in the diet at 0, 4, 32 or 260 ppm (M: 0, 0.3, 2.2 or 19.1; F: 0, 0.4, 3.0 or 24.1 mg/kg/day) for 10 weeks/generation prior to mating (Eigenberg, 1991a). Body weight gains were significantly lower during all phases of the study in F_0 dams at 260 ppm and during lactation in F_1 dams at 260 ppm. The mean body weights were significantly reduced for the F_{1a} pups at 260 ppm from birth (11%) through lactation (21-29%) at 260 ppm. The investigators found in a subsequent cross-fostering study that the low birth weights were not due to a compound-related effect on development, but rather a weight loss that occurred between birth and the time the birth weights were taken which was up to 24 hours later (Eigenberg, 1991b). There was no difference in the mean body weights for the F_{2a} pups at birth, but by day 4 the mean body weight at 260 ppm was reduced by 9%. The mean pup weights were significantly reduced at 260 ppm on days 7, 14, and 21 (14-22%). Maternal food consumption was reduced in both generations at 260 ppm. Tremors were observed in one F_0 dam at 260 ppm and abnormal head tilt was seen in three F_1 dams at 260 ppm. Clinical signs observed in F_{1a} and F_{2a} pups at 260 ppm included cannibalization, bite marks, bruised body, diffuse purple discoloration on head, shoulders and abdomen, dehydration, unkempt appearance and moribundity. The investigators attributed the increased cannibalization, bite marks and bruised bodies to some unknown effect of DEF on the dams based on the cross-fostering study (Eigenberg, 1991b).

Several reproductive parameters were affected at 260 ppm (Table 11). There was a noticeable reduction in the fertility index in the F_1 generation (76% vs. 97% for controls), although it was not statistically significant. This effect was considered toxicologically significant based on a supplemental study in which a similar reduction (83% vs. 90% for controls) was observed in the F_1 generation at 260 ppm (Eigenberg, 1991c). There was a significant increase in gestation length in the F_{2a} litters at 260 ppm which was reproduced in the cross-fostering study (Eigenberg, 1991b). There were also significant reductions in the birth index, live birth index, and viability index in both generations at 260 ppm. The reductions in the birth index and live birth index are probably indirectly related to maternal toxicity. Based on the cross-fostering study, the reductions in the neonatal pup weights and the viability index were also probably due to some unknown effect of DEF on the dams (Eigenberg, 1991b).

Cholinesterase activity was measured in adults at week 8 of premating for each generation and in both adults and pups at the terminal sacrifice. At week 8 of premating, there were significant reductions in the mean plasma ChE activity at 32 ppm (F_0M:67%; F_0F:32%; F_1F:41% of controls) and 260 ppm (F_0M:30%; F_1M:43%; F_0F:9%; F_1F:7% of controls). At the terminal sacrifice, a significant reduction in the mean plasma ChE activity was observed at 4 ppm (F_0F:75% of controls), 32 ppm (F_0M:82%; F_0F&F_1F:28% of controls) and 260 ppm (F_0M:22%; F_1M:32%; F_0F:10%; F_1F:7% of controls). At week 8, there were significant reductions in the mean erythrocyte ChE activity at 4 ppm (F_1M: 91% of controls), 32 ppm (F_0M:65%; F_1M:74%; F_0F:63%; F_1F:72% of controls), and 260 ppm (F_0M:50%; F_1M:57%; F_0F:51%; F_1F:55% of controls). At the terminal sacrifice, the mean erythrocyte ChE activity was also significantly reduced at 4 ppm (F_0F:88%; F_1F:93% of controls), 32 ppm (F_0M:69%; F_1M:72%; F_0F:54%; F_1F:51% of controls) and 260 ppm (F_0M:47%; F_1M:61%; F_0F:48%; F_1F:47%). The mean brain ChE activity was reduced only at 32 ppm (F_0F&F_1F:71% of controls) and 260 ppm (F_0M:63%; F_1M:67%; F_0F&F_1F:19% of controls). There were sex-related differences in ChE activity which were most pronounced at the terminal sacrifice. One

F. REPRODUCTIVE TOXICITY (cont.)

Table 11. The Reproductive Effects of DEF in a Two-Generation Rat Study

Reproductive Effect	Generation	Dose Level (ppm)			
		0	4	32	260
Fertility Index	F_0	90	97	90	90
(%)	F_1	97	93	90	76
Mean Gestation Length	F_{1a}	21.8	22.0	21.9	22.2
(days)	F_{2a}	21.9	22.0	22.0	22.4*
Birth Index	F_{1a}	91	89	90	77*
(%)	F_{2a}	92	91	92	87*
Live Birth Index	F_{1a}	100	97	100	80*
(%)	F_{2a}	99	95	100	87*
Viability Index (day 4)	F_{1a}	96	96	100	90*
(%)	F_{2a}	97	100	97	81*
(day 21)	F_{1a}	100	99	99	83*
	F_{2a}	100	99	100	90*
Mean Pup Weight (day 4)	F_{1a}	7.1	7.1	7.2	6.3*
(g)	F_{2a}	6.8	7.2*	7.0	6.7
(day 21)	F_{1a}	49.5	50.2	50.1	35.2*
	F_{2a}	49.1	49.5	50.2	38.3*

* Significantly different from the control group by the Kruskal-Wallis and Mann-Whitney U test ($p < 0.05$).

explanation for these differences was the higher compound consumption in females during lactation. During lactation, the average compound consumption for females in both generations was approximately twice as high as their consumption during premating and gestation (0.7, 5.5, and 39.2 mg/kg/day at 4, 32, and 260 ppm, respectively). Significant reductions in the mean plasma ChE activity were observed in 21-day-old pups at 260 ppm (F_{1a}M:64%; F_{2a}M:49%; F_{1a}F:62%; F_{2a}F:36% of controls). The mean erythrocyte ChE activity was also significantly reduced (F_{2a}M:75%; F_{1a}F:77%; F_{2a}F:62% of controls). There was also a significant reduction in the mean brain ChE activity in 21-day-old F_{2a} pups at 260 ppm (M&F:85% of controls).

There were no apparent compound-related increases in gross pathological findings in the adults. Sporadic gross ocular lesions (discoloration, opacity, reduced size, abnormal texture and enlargement) were observed in all groups of F_0 and F_1 adults which were attributed to the orbital bleeding technique by the investigator. Possible compound-related retinal degeneration was observed microscopically in two females (one at 4 ppm and the other at 260 ppm) with gross ocular lesions (corneal opacity and enlargement, respectively). The eyes were examined in only a few rats with gross ocular lesions, probably because the effect of DEF on the retina

F. REPRODUCTIVE TOXICITY (cont.)

was not known when this study was conducted. Consequently, a dose-response was not apparent. In the pups, possible compound related effects observed at 260 ppm included cannibalism, discolored livers, uninflated lungs (stillbirths) and empty stomachs (non-suckling).

The reproductive NOEL was 32 ppm (3.0 mg/kg/day) based on the reduction in the fertility, birth, live birth and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, and gross pathological lesions in pups. The parental NOEL was 4 ppm (0.4 mg/kg/day) based on the reduced brain ChE activity (71% of control activity) in F_0 and F_1 females at 32 ppm (3.0 mg/kg/day). This study was considered acceptable by DPR.

G. DEVELOPMENTAL TOXICITY

Summary: Two teratology studies were conducted with DEF, one in rats and the other in rabbits. Both studies administered DEF by oral gavage. These studies met FIFRA guidelines. No treatment-related increases in embryotoxicity, fetal malformations or variations were observed in rats and rabbits exposed to DEF by the oral route. Maternal effects included brain ChE inhibition and reduced body weight gain. The maternal NOELs were 7 and 3 mg/kg/day for rats and rabbits, respectively.

In addition, an inhalation teratology study was conducted in which mice and rats were exposed to vapors of nBM. This study did not meet FIFRA guidelines. An increased post-implantation loss was observed in mice exposed to vapors of nBM. The total number of malformations was also higher, although not on a litter basis. The developmental NOEL for nBM was 10 ppm (17 mg/kg/day). The maternal effects included increased mortalities, reduced body weight gain, and clinical signs. The maternal NOEL for nBM in mice was also 10 ppm. There were no treatment-related increases in developmental or maternal effects in rats exposed to vapors of nBM.

Gavage-Rat

In a teratology study, DEF (98%) was administered to 33 mated female Sprague-Dawley rats/group by oral gavage in 0.5% carboxymethylcellulose at 0, 1, 7 or 28 mg/kg/day on gestation days 6 to 15 (Kowalski *et al.*, 1986). Excessive salivation was observed in two dams at 28 mg/kg/day on treatment days 3 and 6 (gestation days 9 and 12). There was a significant reduction in the mean body weight gain of the 28 mg/kg/day group. The mean plasma and erythrocyte ChE activity in the dams was significantly reduced at 7 mg/kg/day (42 and 29% of controls, respectively) and 28 mg/kg/day (25 and 13% of controls, respectively) on day 16. Although the mean maternal brain ChE activity remained significantly reduced (54% of control) on day 20 at 28 mg/kg/day, fetal brain ChE activity was unaffected. No treatment-related teratogenic or other developmental effects were seen. The maternal NOEL was 7 mg/kg/day based on the brain ChE inhibition and reduced body weight gain. The developmental NOEL was greater than or equal to 28 mg/kg/day, the highest dose tested. This was an acceptable study to DPR.

Gavage-Rabbit

Groups of 17 mated female American Dutch rabbits were given DEF (98%) by oral gavage in carboxymethylcellulose at 0, 1, 3, or 9 mg/kg/day on days 7 to 19 of gestation

(Clemens *et al.*, 1987). Although control animals gained 150 g on average from gestation days 7 to 21, animals at 9 mg/kg/day gained no weight on average during this time. The animals at 9 mg/kg/day also tended to consume less food during the treatment period, although the difference was not statistically significant. The mean plasma ChE activity was significantly reduced at 1 mg/kg/day (60% of controls), 3 mg/kg/day (46% of controls), and 9 mg/kg/day (33% of controls) on day 20. There was also a significant reduction in erythrocyte ChE activity at 1 mg/kg/day (30% of controls), 3 mg/kg/day (15% of controls), and 9 mg/kg/day (7% of controls) on day 20. The slight reduction in the mean brain ChE activity (95% of controls) at 9 mg/kg/day was not statistically significant. There was no treatment-related increase in embryotoxicity, fetal malformations or variations. The maternal NOEL was 3 mg/kg/day based on no body weight gain during exposure. The developmental NOEL was greater than or equal to 9 mg/kg/day, the highest dose tested. DPR found this study acceptable.

N-Butyl Mercaptan

Inhalation-Mouse

In an inhalation teratology study, 25 pregnant female mice/dose were exposed to vapors of nBM (97.5%) for 6 hrs/day at 0, 10, 68 or 152 ppm (actual; 0, 17, 113 or 252 mg/kg/day)[6] during gestation days 6-16 (Thomas *et al.*, 1987). Seventeen mice at 68 and 152 ppm died. One dam at 152 ppm had limb paralysis and spasmodic respiratory appearance. Emaciation, unkempt appearance, lethargy and red/brown perianal staining were seen in dams at 68 and 152 ppm. There was a reduction in the terminal maternal body weights (>10%) and an increase in postimplantation losses at 68 and 152 ppm. The total number of fetuses with malformations (which included cleft palate, open eye, exencephaly, hydrocephaly, vertebral anomalies and bent bones) was also significantly higher at 68 ppm, although there was no significant difference in the number of malformations/group on a litter basis. Four of the 5 fetuses with cleft palate at 68 ppm occurred in two litters in which there was evidence of both maternal and fetal toxicity based on maternal weight loss and lower fetal weights. The maternal NOEL for nBM was 10 ppm (17 mg/kg/day) based on the mortality, reduced weight gain and clinical signs. The developmental NOEL was also 10 ppm based on the increased postimplantation losses and malformations. This study was only available as a published report and, therefore, it is not known if it met FIFRA guidelines.

Inhalation-Rat

Thomas *et al.* (1987) also exposed 25 pregnant female rats/dose to vapors of nBM (97.5%) at 0, 10, 68 or 152 ppm (actual; 0, 9, 60 or 135 mg/kg/day)[7] for 6 hr/day during gestation days 6-19 (Thomas *et al.*, 1987). There were no mortalities or significant treatment-related clinical signs. There was no evidence of developmental toxicity. The maternal and developmental NOELs in rats were greater than 152 ppm (135 mg/kg/day), the highest dose tested.

---

[6] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a mouse was assumed to be 0.45 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979).

[7] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a rat was assumed to be 0.24 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979).

H. NEUROTOXICITY

Summary: Numerous neurotoxicity studies have been conducted in which hens were exposed to DEF by the intraperitoneal, subcutaneous, inhalation, oral, or dermal routes (Tables 12 and 13). Due to the large number of studies, most of these studies will only be discussed briefly. Most of these studies were available as published reports and did not follow the standard protocol recommended in the FIFRA guidelines. One subchronic dermal neurotoxicity study did meet FIFRA guidelines. Delayed neuropathy was observed in acute and subchronic studies in which hens were exposed by the inhalation, oral, and dermal routes. In addition, cholinergic signs and other effects described as "late acute" effects were seen; however, the late acute effects were only observed with oral exposure in hens. The late acute effects were attributed to the hydrolysis of DEF to nBM which was found in the excreta of hens administered DEF orally. In subsequent studies, it was shown in hens that nBM causes erythrocyte deformation and lysis through the inhibition of glucose-6-phosphate dehydrogenase. With acute oral exposure in hens, the OPIDN occurred at approximately the same dose levels or higher than the cholinergic and late acute effects. However, the distinction between the cholinergic, late acute, and delayed neurotoxic effects was blurred in some studies because some effects such as leg weakness and unsteadiness were common to all syndromes and could only be separated based on their time of onset. There is also some uncertainty about the dose levels at which cholinergic signs occurred because many of the studies provided insufficient information about the incidence of cholinergic effects to accurately determine a NOEL. The lowest NOEL with acute inhalation exposure to DEF was less than 43 mg/kg based on mild cholinergic signs (Thyssen and Schilde, 1976a). With acute oral exposure, the lowest NOEL was 50 mg/kg based on death, unspecified toxic signs, late acute effects, and OPIDN (Thyssen, 1976; Abou-Donia *et al.*, 1979a). The lowest acute dermal NOEL was 100 mg/kg based on mild cholinergic signs and OPIDN (Abou-Donia *et al.*, 1984; Abdo *et al.*, 1983a). In the subchronic studies in hens, the OPIDN generally occurred at lower doses than the cholinergic or late acute effects. The lowest NOEL with subchronic inhalation exposure to DEF was 3.6 mg/kg/day based on mild cholinergic signs and OPIDN (Thyssen and Schilde, 1978a). The lowest subchronic oral NOEL was 0.1 mg/kg/day based on OPIDN (Abou-Donia *et al.*, 1979b). With subchronic dermal exposure to DEF, the lowest NOEL was 2.6 mg/kg/day based on OPIDN (Sheets, 1991b).

Animal Studies

Parenteral Studies

Casida and coworkers (1963) first reported evidence of OPIDN when chickens developed ataxia 10-14 days after 7-10 daily intraperitoneal injections of DEF at 100 mg/kg/day with and without atropine protection. A similar study conducted by Baron and Johnson (1964) reported muscle weakness, ataxia, paralysis, and degenerative lesions in the sciatic nerve and spinal cord in hens after 3-15 intraperitoneal injections of DEF at 50 and 100 mg/kg/day. Johnson (1970 a&b) also reported evidence of OPIDN in hens after a single subcutaneous injection of DEF at approximately 1,000 mg/kg with an onset around day 8. NTE activity, measured in the brain of two hens 17 and 24 hours after dosing, was reduced to 23% of the control activity.

H. NEUROTOXICITY (cont.)

Table 12. Acute Neurotoxicity Studies for DEF with Hens

Dosage	Hens/Dose	Effect	NOEL (mg/kg)	LOEL (mg/kg)	Ref.[a]
		Inhalation[b]			
391, 878 or 1,585 mg/m^3, single 4-hr exposure	5	Leg weakness, drowsiness, inactivity, breathing disorders	----	43	1
		Ataxia, paralysis (onset day 15), degeneration of sciatic nerve	97	174	
62, 145 or 246 mg/m^3, 4-hrs/day for 5 days	10	Ataxia (onset day 16-18) (paralysis, nerve degeneration at 27 mg/kg)	6.8	16	1
		Leg weakness, drowsiness, inactivity, breathing disorders	16	27	
		Subcutaneous			
200 or 1,060 mg/kg	2-3	Ataxia, paralysis (onset day 8)	200	1060	2
220 or 1,100 mg/kg	2	Ataxia (onset not reported)	220	1010	3
		Oral			
0, 50 - 500 mg/kg, gavage	10	Death and unspecified toxic effects	50	100	4
0, 50 - 1,000 mg/kg, one capsule	3	Late acute effects (onset day 2-14), ataxia (onset day 4-17)	50	100	5
0, 100 - 1,000 mg/kg, one capsule	5	Ataxia (onset not reported), peripheral demyelination (1 hen)	----	100	6
		Dermal			
0.5, 1 or 2 ml/kg, dorsal skin	5	Impaired general health, ataxia, paralysis (onset week 2-3)	~500	~1000	7
0, 400 or 1,000 mg/kg, comb	3	Brain ChE inhibition (74% of control), ataxia (onset day 6-11) (nerve degeneration at 1,000 mg/kg)	----	400	5
100 - 1,000 mg/kg, neck	5	Ataxia, paralysis (onset day 9-10)	100	250	8
		Unspecified mild cholinergic signs	250	500	
0, 100 - 1,000 mg/kg, back of neck	5	Unspecified mild cholinergic signs, ataxia, paralysis (onset not reported)	100	250	6

[a] References: 1. Thyssen and Schilde, 1976a; 2. Johnson, 1970a; 3. Johnson, 1970b; 4. Thyssen, 1976; 5. Abou-Donia *et al.*, 1979a; 6. Abou-Donia *et al.*, 1984; 7. Thyssen and Schilde, 1976b; 8. Abdo *et al.*, 1983a.

[b] Air concentrations were converted to mg/kg by assuming a respiratory rate of 0.11 m^3/kg/4 hrs (Dejours *et al.*, 1970).

H. NEUROTOXICITY (cont.)

Table 13. Subchronic Neurotoxicity Studies for DEF in Hens

Dosage	Hens/Dose	Effect	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Ref.[a]
		Inhalation[b]			
8, 21 or 84 mg/m^3, 6 hr/day, 5 day/wk, 3 wks	10	Decreased preening, lethargy, ataxia, paralysis (onset week 4), degeneration of sciatic nerve (1 hen)	3.6	14.3	1
		Intraperitoneal			
100 mg/kg/day, 7 or 10 days	NR	Ataxia (onset day 10-14)	----	100	2
50 or 100 mg/kg/day, 3 to 15 days	5-28	Muscle weakness, ataxia, paralysis, nerve degeneration	----	50	3
		Oral			
50, 100 or 150 mg/kg/day, 4-15 days	1-7	Unspecified degenerative lesions in spinal cord and sciatic nerve (1 hen)	100	150	3
0, 100, 250 or 500 ppm, 30 days, diet	6	Focal liquefication of brain	34[c]	87	4
0, 25 - 400 ppm, 30 days, diet	10	Reduced food consumption, perivascular CNS[d] & PNS inflammation	6.1	10.9	5
0, 0.1 - 80 mg/kg/day, capsule, 90 days	5	Ataxia (onset day 30) Late acute effects (onset day 2-5), paralysis (onset day 19-30), nerve degeneration	0.1 10	0.5 20	6
3 - 40 mg/kg/day, capsule, 91-97 days	3-4	Death, ataxia, paralysis (onset day 10-26)	5-6	38-40	7
		Dermal			
0, 0.01 - 1 ml/kg/day, 6 hr/day, 5 days/wk, 3 wks, axilla	8	Ataxia, paralysis (onset week 3) Unspecified cholinergic signs	~30 ~100	~100 ~300	8
0, 20 or 40 mg/kg/day, 90 days, comb	3	Ataxia (onset day 8-22)	----	20	6
6 - 16 mg/kg/day, 91-101 days, comb	3	Ataxia (onset day 76-100); skin: thickening of keratin and epidermis, collagen deposition, inflammation	----	6-8	7
0, 2.6, 11, 42 mg/kg/day, 5 day/wk, 13 wks, comb	12	Axonal degeneration	2.6	11	9*

[a] References: 1. Thyssen and Schilde, 1978a; 2. Casida *et al.*, 1963; 3. Baron and Johnson, 1964; 4. Harris, 1965; 5. Thyssen *et al.*, 1977; 6. Abou-Donia *et al.*, 1979b; 7. Hansen *et al.*, 1982; 8. Thyssen and Schilde, 1978b; 9. Sheets, 1991b.

[b] Air concentrations were converted to mg/kg by assuming a respiratory rate of 0.17 m^3/kg/6 hrs (Dejours *et al.*, 1970).

[c] Using the mean food consumption for each group from the study and assuming a body weight of 2 kg.

[d] CNS = central nervous system; PNS = peripheral nervous system

* Acceptable study based on FIFRA guidelines

H. NEUROTOXICITY (cont.)

Inhalation Studies

Three inhalation studies were conducted in hens with the exposure ranging from a single 4-hr exposure to daily 6-hr exposures, 5 days/week for 3 weeks (Thyssen and Schilde, 1976a; Thyssen and Schilde, 1978a). Evidence of OPIDN (ataxia, paralysis and nerve degeneration) was observed in all 3 studies. Compared to cholinergic signs, the development of OPIDN appeared to be especially sensitive to repeated inhalation exposure. With a single inhalation exposure, the acute LOEL for OPIDN was 4-fold higher (878 mg/m^3 or 174 mg/kg[8]) than for cholinergic signs (391 mg/m^3 or 43 mg/kg). However, with 5 consecutive inhalation exposures, the LOEL for OPIDN was nearly 2-fold lower (145 mg/m^3 or 16 mg/kg/day) than for cholinergic signs (246 mg/m^3 or 27 mg/kg/day). The subchronic NOEL for OPIDN was 21 mg/m^3 (3.6 mg/kg/day)[9]. None of these studies met FIFRA guidelines.

Oral Studies

Several of the initial oral studies for DEF suggested that OPIDN was not easily produced by this route. Baron and Johnson (1964) did not observe any evidence of OPIDN in hens when DEF was administered by oral gavage at 50-150 mg/kg for 4-15 days with the possible exception of one hen. In a 30-day feeding study, there was equivocal histological evidence of OPIDN (demyelination of the spinal cord at 100 and 250 ppm only; focal liquefication of the brain at 250 and 500 ppm) in 1 of 6 hens per dose when fed DEF at 100, 250 or 500 ppm (Harris, 1965). Thyssen (1976) found no clinical or histological evidence of OPIDN when hens were administered DEF by oral gavage at 300 mg/kg twice with a 21-day interval between each dose. Equivocal histological evidence of OPIDN (perivascular CNS and PNS inflammation) was also seen in another 30-day feeding study in which hens were fed DEF at 0, 25, 50, 100, 200 or 400 ppm (Thyssen *et al.*, 1977).

Evidence of OPIDN was observed in four other oral neurotoxicity studies with hens (Abou-Donia *et al.*, 1979a&b; Abou-Donia *et al.*, 1984; Hansen *et al.*, 1982). An acute NOEL of 50 mg/kg/day was established for OPIDN in one study (Abou-Donia *et al.*, 1979a). A significantly lower NOEL of 0.1 mg/kg/day was observed in an oral subchronic study with hens based on mild ataxia (Abou-Donia *et al.*, 1979b). However, there was limited histological evidence of delayed neuropathy with oral exposure even at high doses (Table 14). Unequivocal histological lesions in the spinal cord or peripheral nerve were not observed in this study until the dose was increased to 20 mg/kg. At 80 mg/kg/day, 1 out of 5 hens had unequivocal lesions and 2 out of 5 hens had equivocal lesions in the spinal cord indicative of OPIDN. The equivocal lesions were ones Abou-Donia *et al*. (1979b) suggested could be early signs of delayed neuropathy, but because they were occasionally observed in controls he could not be certain. However, there was no dose-response relationship in the incidence of the equivocal lesions with oral exposure. More likely these lesions were age-related because the birds were relatively old (19 months). Abou-Donia *et al*. (1979b) did not report the incidence of the histological lesions at 0.1 mg/kg/day or in the controls, making interpretation of the equivocal lesions difficult. Hens receiving 20-80 mg/kg/day orally, developed severe ataxia, some became paralyzed and nearly

---

[8] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a chicken was assumed to be 0.11 m^3/kg/4 hrs (Dejours *et al.*, 1970).

[9] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a chicken was assumed to be 0.17 m^3/kg/6 hrs (Dejours *et al.*, 1970).

H. NEUROTOXICITY (cont.)

Table 14. Incidence of Histological Lesions in Spinal Cord or Peripheral Nerve[a]

Dose mg/kg/day	Oral[b]			Dermal[c]		
	Positive	Equivocal	Negative	Positive	Equivocal	Negative
80	1	2	2	—	—	—
40	0	1	4	3	0	0
20	1	0	4	0	1	2
10	0	1	4	—	—	—
5	0	1	4	—	—	—
2.5	0	0	5	—	—	—
1	0	2	3	—	—	—
0.5	0	2	3	—	—	—

[a] Abou-Donia *et al.*, 1979b
[b] Five hens exposed by the oral route per dose level
[c] Three hens exposed by the dermal route per dose level

all died from late acute effects within the first few weeks of exposure. Abou-Donia *et al*. (1979b) suggested that more histological lesions would have been seen with oral exposure if the hens had lived longer. However, if there was enough nBM to kill these hens, there probably was significantly less DEF available to produce delayed neuropathy. Moreover, the nBM is probably responsible for the ataxia and paralysis with oral exposure because of the limited evidence of delayed neuropathy even at lethal doses. Abou-Donia *et al*. (1979b) and Fairchild and Stokinger (1958) reported that nBM caused muscle weakness, incoordination, paralysis, CNS depression and cyanosis all of which could affect gait. NTE activity was not measured in the Abou-Donia *et al.* (1979b) study which would have helped in the interpretation of the clinical and histopathological findings. A LOEL of 20 mg/kg/day for OPIDN would be more consistent with the LOEL reported for a similar oral subchronic neurotoxicity study in which death, paralysis, and nerve degeneration were observed in hens at approximately 40 mg/kg/day (Hansen *et al.*, 1982). None of the oral neurotoxicity studies for DEF met FIFRA guidelines because of inadequate exposure duration, inadequate number of hens per group, age of hens, no analysis of test article or dosing material, inadequate or no histopathology data or no positive controls.

One explanation for the reduced incidence of OPIDN in hens administered DEF by the oral route may be the hydrolysis of DEF to nBM in the gastrointestinal tract. Abou-Donia *et al.* (1979a) first reported "late acute" effects in hens administered a single capsule containing DEF at 100 mg/kg or higher. The hens exhibited leg weakness, unsteadiness and a yellowish watery liquid around the mouth by the second day after dosing. Their condition progressively worsened with malaise, general muscle weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors and loss of breath which were not responsive to atropine therapy. Just prior to death, their combs became dark and droopy. These late acute effects were distinguished from those associated with OPIDN in that the onset of death was earlier (2-14

days after dosing) and no histological lesions were found in the sciatic nerve. nBM was identified by mass spectrometry in the excreta of hens administered DEF orally. These investigators tested the possibility that these effects were due to nBM by administering hens a single capsule containing nBM (98%) at 100, 400 or 1,000 mg/kg. The hens at 400 and 1,000 mg/kg developed clinical signs similar to the late acute effects observed when DEF was administered orally; however, the onset of signs was earlier (6-12 hrs after administration). No degenerative changes in the sciatic nerve were present in any of the hens treated with nBM. Furthermore, there was a slight increase or no change in brain and plasma ChE activity of hens exposed to nBM. The half-life of nBM was estimated to be 8 days based on the plasma levels.

A mechanism of action for nBM toxicity was proposed by Abdo *et al.* (1983b) who found an increase in Heinz bodies and extensive erythrocyte deformation and lysis 24 to 48 hrs after hens were given nBM in capsules at 500 mg/kg. Methemoglobin levels were significantly higher in the treated birds while the hemoglobin concentration, packed cell volume, erythrocytes and glucose-6-phosphate dehydrogenase (G-6-PD) activity were significantly lower. The time course for disappearance of hematological changes and late acute effects was similar and the investigators suggested that the inhibition of G-6-PD by nBM led to the hematological changes. This enzyme is required to regenerate nicotinamide adenine dinucleotide phosphate (NADPH) which is needed for the reduction of glutathione. Decreased levels of reduced glutathione resulted in the denaturation of hemoglobin (i.e., formation of methemoglobin and Heinz bodies), coagulation of surface proteins on erythrocytes leading to deformation, and eventual cell lysis. The investigators concluded the late acute effects observed after oral administration of DEF were directly related to the hematological changes.

Dermal Studies

Evidence of OPIDN was observed in nearly all of the acute dermal neurotoxicity studies in hens (Thyssen and Schilde, 1976b; Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984; Abdo *et al.*, 1983a; Thyssen and Schilde, 1978b; Abou-Donia *et al.*, 1979b; Hansen *et al.*, 1982; Sheets, 1991b). The lowest acute NOEL for OPIDN by the dermal route was 100 mg/kg (Abdo *et al.*, 1983a; Abou-Donia *et al.*, 1984). None of the acute dermal neurotoxicity studies met FIFRA guidelines.

There was evidence of OPIDN was found in a number of subchronic dermal neurotoxicity studies in hens (Thyssen and Schilde, 1978b; Abou-Donia *et al*, 1979b; Hansen *et al.*, 1982; Sheets, 1991b). It is interesting to compare the findings with oral and dermal exposure from the Abou-Donia *et al.* (1979b) study at 40 mg/kg/day (Table 14). With oral exposure, 4 out of 5 hens had no lesions and 1 out of 5 hens had an equivocal lesion in the spinal cord suggestive of OPIDN. By contrast, with dermal exposure unequivocal lesions of OPIDN were observed in the spinal cord of all 3 hens. While all the hens administered DEF at 40 mg/kg/day by the oral route died within the first few weeks of exposure, none of the hens administered DEF at this same dose level by the dermal route died despite developing clear evidence of delayed neuropathy. A NOEL could not be established for delayed neuropathy with dermal exposure because animals at the lowest dose developed ataxia which, unlike with oral exposure, was probably related to OPIDN since significantly less nBM would likely be formed with this route of exposure.

Only one subchronic dermal study conducted by Sheets (1991b) was acceptable to DPR based on the FIFRA guidelines. In this study, 12 white leghorn hens/group were administered

H. NEUROTOXICITY (cont.)

DEF (97.7%) topically to the comb at 0, 2.6, 11, and 42 mg/kg/day for 5 days/week for 13 weeks. Whole blood cholinesterase activity was significantly reduced at 2.6 mg/kg/day (53% of controls), 11 mg/kg/day (43% of controls), and 42 mg/kg/day (43% of controls). Decreased motor activity and ataxia were observed in all hens at 42 mg/kg/day with an onset between days 12 and 39. There was a high background rate for axonal degeneration probably due to the age of the birds which were older (17 months) than recommended by FIFRA guidelines (8-14 months), thus making interpretation of the histological findings difficult. The axonal degeneration was identical to that encountered in older hens that have had contact with vaccines or other exogenous viral exposure, such as Marek's disease. There was a statistically significant increase in the severity of the axonal degeneration at 42 mg/kg/day. Although not statistically significant, there was a slight increase in the severity and incidence of axonal degeneration at 11 mg/kg/day. There were only two instances of mild ataxia on days 71 and 80 in 1 of 12 hens at 11 mg/kg/day, suggesting that most of axonal degeneration was age-related. DPR made the health protective assumption that the axonal degeneration was treatment-related and set the NOEL at 2.6 mg/kg/day. The LOEL in this study is consistent with the LOELs established in two other subchronic dermal neurotoxicity studies (Abou-Donia *et al.*, 1979b; Hansen *et al.*, 1982); however, the NOEL was the lowest subchronic NOEL observed for OPIDN by the dermal route.

A study was conducted to evaluate the neurotoxic effects of DEF in hens from normal field use. Scaleless hens were exposed to varying levels of DEF over a 7-hour period based on their proximity to a cotton field that was sprayed with DEF by a rig (Wilson *et al.*, 1980). Dermal exposure was estimated by measuring residues on mylar sheets placed next to the hens. The estimated dermal exposure ranged from 0.0092 $\mu g/cm^2$ in unsprayed rows of cotton to 47.8 $\mu g/cm^2$ on the rig near the hens exposed for one day. The dermal exposure for hens exposed daily in treated rows for a week was estimated to be 108 $\mu g/cm^2$. Air concentrations of DEF were also measured and ranged from 0.111 mg/m^3 in untreated rows to 13.8 mg/m^3 near the rig. None of the hens exhibited ataxia or other signs of OPIDN.

Human Studies

Kilgore *et al.* (1984) conducted a study with pesticide workers before and after a 7-week exposure period to DEF in which medical examinations and neuro-psychological tests were performed. No significant effects were found including cholinesterase inhibition. Another worker exposure study was conducted by Lotti *et al.* (1983) in which pesticide workers were monitored before and after the normal use season. No differences were detected between pre- and post-exposure electromyographs and nerve conduction tests. The whole blood and plasma ChE levels were all within 25% of pre-exposure levels. However, the lymphocyte NTE activity was reduced to between 40 and 60% of pre-exposure levels between days 25 and 30 of exposure. Neither of these studies provided sufficient information to accurately estimate total DEF exposure.

IV. RISK ASSESSMENT

A. HAZARD IDENTIFICATION

Acute Toxicity

The effects observed in experimental animals after acute exposure to DEF are summarized in Table 15. In addition to the effects observed in the LD_{50}/LC_{50} studies and the acute neurotoxicity studies, some findings observed in the 90-day inhalation, 21-day dermal and developmental toxicity studies were also considered as acute effects. These include signs observed within the first few days of exposure and any fetal effects assuming they were the result of a single exposure. The clinical signs observed after acute exposure to DEF were primarily neurological. Cholinergic signs were seen in most laboratory animals after acute exposure to DEF by various routes. Hypothermia was observed in rats, mice and guinea pigs when DEF was administered by the oral, intraperitoneal and intravenous route (Ray, 1980; Ray and Cunningham, 1985). The investigators suggested a selective action on a central thermogenic control process may be involved. Other research indicates that the hypothermia associated with organophosphates is due to central AChE inhibition because hypothermia is antagonized by centrally active antiChE drugs, such as atropine, but not by peripherally active antiChE drugs, such as 2-PAM (Kenley *et al.*, 1982). Organophosphate-induced delayed neuropathy (OPIDN) was observed in hens in the form of ataxia, paralysis, and nerve degeneration approximately 2-3 weeks after acute exposure to DEF by inhalation, oral or dermal routes. There was no evidence of DEF-induced delayed neuropathy in other species tested; however, rodents generally are less susceptible to OPIDN (Abou-Donia, 1981; Somkuti *et al.*, 1988; De Bleeker *et al.*, 1992). Sensitivity in rodents appears to vary depending on strain and age (Padilla and Veronesi, 1988; Veronesi *et al.*, 1991; Moretto *et al.*, 1992; Inui *et al.*, 1993).

Other effects described as “late acute” effects were also seen in hens; however, the late acute effects were only observed with oral exposure (Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984). Abou-Donia and coworkers attributed these late acute effects to nBM which was found in the excreta of hens after oral exposure, presumably from the hydrolysis of DEF in the gut. In subsequent studies, it was shown that in hens nBM causes erythrocyte deformation and lysis through the inhibition of glucose-6-phosphate dehydrogenase (Abdo *et al*, 1983b). Clinical signs similar to the late acute effects in hens have not been described in other species administered DEF, but changes in erythrocyte morphology were seen in rabbits administered DEF (route not indicated) at 242 mg/kg (Mirakhmedov *et al.*, 1989). In addition, mice, rats, and dogs had reductions in erythrocyte counts, hematocrits and hemoglobin after long-term exposure to DEF in the diet (Hayes, 1989; Christenson, 1991; Christenson, 1992). Similar hematological changes were also seen at the termination of a 90-day inhalation study in rats, presumably from either degradation of DEF in the chamber or normal tissue metabolism of DEF (Pauluhn, 1992).

After acute oral exposure in hens, OPIDN occurred at approximately the same dose levels or higher than the cholinergic and late acute effects. However, the distinction between the cholinergic, late acute, and delayed neurotoxic effects was blurred in some hen studies because some effects such as leg weakness and unsteadiness were common to all syndromes and could only be separated based on their time of onset. There is also some uncertainty about the dose levels at which cholinergic signs occurred because many of the hen studies provided insufficient information about the incidence of cholinergic effects to accurately determine a NOEL. In mammals, cholinergic signs were the primary effects observed after acute exposure

A. HAZARD IDENTIFICATION (cont.)

Table 15. Acute Adverse Effects of DEF and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL mg/kg	LOEL mg/kg	Ref.[a]
		Inhalation[b]			
Rat	Single, 4-hr, nose only	Death, cholinergic signs, red turbinates, firm zones in lungs	------	254	1*
Rat	Single, 4-hr, nose only	Decreased preening, lethargy	12.3	20.8	2
Rat	13 weeks, 6 hr/day, 5 day/wk	Reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing, increased startle response (onset days 1-3)	2.9	14.3	3*
Hen	Single, 4-hr	Leg weakness, drowsiness, inactivity, breathing disorders	------	43	4
	5 Days, 4-hr	Ataxia	6.8	16	
		Intraperitoneal			
Rat	Single, injection	Hypothermia	------	20	5
		Oral			
Rat	Single, gavage	Cholinergic signs	------	192	6*
Rat[c]	**9 Days, gavage**	**Excessive salivation (onset day 3)**	**7**	**28**	**7***
Hen	Single, gavage	Death, unspecified toxic effects	50	100	8
Hen	Single, capsule	"Late acute" effects, ataxia	50	100	9
Hen	Single, capsule	Ataxia, peripheral demyelination	------	100	10
		Subcutaneous			
Hen	Single, injection	Ataxia, paralysis	200	1060	11
Hen	Single, injection	Ataxia	220	1010	12
		Dermal			
Rabbit	Single, 24-hr	Cholinergic signs, erythema	------	500	13*
Rabbit	6 hr/day, 5 day/wk, 3 weeks	Muscle fasciculations (onset day 2)	11	29	14*
Hen	Single, 24-hr	Impaired general health, ataxia, paralysis	500	1000	15
Hen	Single	Brain ChE inhibition, ataxia (nerve degeneration at 1,000 mg/kg)	------	400	9
Hen	Single	Ataxia, paralysis	100	250	16
Hen	Single	Ataxia, paralysis, cholinergic signs	100	250	10

[a] References: 1. Warren, 1990; 2. Thyssen, 1978a; 3. Pauluhn, 1992, 4. Thyssen and Schilde, 1976a; 5. Ray, 1980; 6. Sheets, 1991a; 7. Kowalski *et al.*, 1986; 8. Thyssen, 1976; 9. Abou-Donia *et al.*, 1979a; 10. Abou-Donia *et al.*, 1984; 11. Johnson, 1970a; 12. Johnson, 1970b; 13. Sheets and Phillips, 1991; 14. Sheets *et al.*, 1991; 15. Thyssen and Schilde, 1976b; 16. Abdo *et al.*, 1983a.

[b] Estimated assuming a respiratory rate of 0.16 and 0.11 m^3/kg/4 hrs for a rat and hen, respectively.

[c] Developmental toxicity study: All fetal effects were considered acute effects; however, only maternal effects observed within the first few days of exposure were considered acute effects.

* Acceptable study based on FIFRA guidelines.

to DEF; however, the dose levels were too high in the standard LD_{50}/LC_{50} tests to establish a NOEL. Acute NOELs were established for DEF in three studies that met FIFRA guidelines. A NOEL of 2.9 mg/kg was observed for acute effects in a 90-day inhalation study (Pauluhn, 1992). The respiratory uptake and absorption was assumed to be 100%. An acute NOEL of 7 mg/kg/day was established in a rat developmental toxicity study in which excessive salivation was observed in the dams at 28 mg/kg on treatment day 3 (Kowalski *et al.*, 1986). After correcting for oral absorption (70%), the adjusted NOEL was 4.9 mg/kg. An acute NOEL of 11 mg/kg was also established in a 21-day dermal toxicity study in which muscle fasciculations were observed in 9 of 10 rabbits at 29 mg/kg/day on day 2 (Sheets *et al.*, 1991). After correcting for dermal absorption (47.5%), the adjusted NOEL was 5.2 mg/kg. Despite evidence in hens that the metabolism of DEF may be different with oral exposure, the NOELs were relatively similar in these three mammalian studies after adjusting for absorption. Greater importance was placed on the oral and dermal studies because these were the main routes of exposure with dietary and occupational exposure, respectively. Consequently, the developmental toxicity study was selected for characterizing the risk for adverse health effects from acute occupational and dietary exposure to DEF based on excessive salivation in pregnant rats. The critical NOEL was 4.9 mg/kg, after adjusting for oral absorption.

Mild dermal and ocular irritation were observed with exposure to technical grade DEF. However, the formulation caused severe eye irritation and was corrosive to the skin (Crawford and Anderson, 1972a; Sheets and Fuss, 1991; Sheets and Phillips, 1992a). A NOEL for the dermal irritation was estimated to be 8.3 mg formulation/cm^2 by dividing the amount of DEF applied to the site (0.5 ml per 6 cm^2) by an uncertainty factor of 10 to extrapolate from a LOEL to a NOEL. This critical NOEL was selected for characterizing the risk for local effects from acute dermal exposure to DEF formulations.

Subchronic Toxicity

The effects of subchronic exposure to DEF in experimental animals are summarized in Table 16. Included in this summary are some maternal effects observed in developmental toxicity studies after the first few days of exposure and all effects observed in reproductive toxicity studies. Not included in this table were two neurotoxicity studies in which hens were given daily intraperitoneal injections at 50 and 100 mg/kg for 5 to 15 days (Casida *et al.*, 1963; Baron and Johnson, 1964). Baron and Johnson (1964) also observed OPIDN in hens administered DEF by oral gavage at 50-150 mg/kg/day for 5 to 15 days. These two studies were not used because NOELs were not established, and they had major deficiencies including inadequate number of animals and inconsistent exposure periods within and between dose levels. Also, not included was a 3-month feeding study in rats and dogs (Root and Doull, 1966). A NOEL of 5 ppm (0.25 and 0.125 mg/kg/day for rats and dogs, respectively) was reported for both species, but the effects seen at the LOEL were not reported. This study had other major deficiencies such as no summary of body weights, food consumption, hematology, clinical chemistry or pathological lesions.

Signs of OPIDN were the most frequent effects seen in hens and usually occurred at lower doses than the cholinergic signs, regardless of the route of exposure. In mammals, brain ChE inhibition and cholinergic signs were some of the more sensitive endpoints. Other effects observed in rodents with subchronic exposure include reduced weight gain, reduced food consumption, hematological changes, impaired retinal function, pale retinal fundus, fatty droplets in the adrenal cortex, and increased adrenal weights. Several reproductive effects

A. HAZARD IDENTIFICATION (cont.)

Table 16. Subchronic Adverse Effects of DEF and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL mg/kg/day	LOEL mg/kg/day	Ref.[a]
		Inhalation[b]			
Rat	6 hr/day, 5 day/wk, 3 weeks, nose only	Brain ChE[c] inhibition (73%), decreased preening, lethargy, inflammation in lung	1.7	7.7	1
Rat	6 hr/day, 5 day/wk, 2 weeks, nose only	Cholinergic signs, brain ChE inhibition (61%)	3.2	15	2
Rat	6 hr/day, 5 day/wk, 13 weeks, nose only	Cholinergic signs, hematological changes, brain ChE inhibition (60%), impaired retinal function, pale retinal fundus, fatty droplets in adrenals, inc. adrenal wts.	2.9	14.3	3*
Hen	6 hr/day, 5 day/wk, 3 weeks	Dec. preening, lethargy, ataxia, paralysis, nerve degeneration	3.6	14.3	4
		Oral			
Mouse	8 weeks, feed	Brain ChE inhibition (74%)	40	140	5
Rat[d]	9 days, gavage	Reduced maternal weight gain and maternal brain ChE inhibition (54%)	7	28	6*
Rat[e]	Diet, 2-gen., 10 wk/gen.	Parental: Brain ChE inhibition (F:71%) Reproductive: Reduced fertility, birth and viability indices, increased gestation length, reduced pup weights, cannibalism of pups, discolored pup livers	0.4 3.0	3.0 24.7	7*
Rabbit[d]	12 days, gavage	Reduced maternal weight gain	3	9	8*
Hen	30 days, feed	Focal liquefication of brain	34	87	9
Hen	30 days, feed	Reduced food consumption, neurohistological lesions	6.1	10.9	10
Hen	90 days, capsule	Ataxia	0.1	0.5	11
Hen	91-97 days, capsule	Death, ataxia, paralysis	5-6	38-40	12
		Dermal			
Rabbit	**6 hr/day, 5 day/wk, 3 weeks**	**Muscle fasciculations, brain ChE inhibition (85-86%), skin lesions**	**2**	**11**	**13***
Hen	6 hr/day, 5 day/wk, 3 weeks	Ataxia, paralysis	~30	~100	14
Hen	90 days	Ataxia	--	20	11
Hen	91-101 days	Ataxia, dermal irritation	--	6-8	12
Hen	13 weeks, 5 day/wk	Axonal degeneration	2.6	11	15*

[a] References: 1. Thyssen, 1978b; 2. Pauluhn, 1991; 3. Pauluhn, 1992; 4. Thyssen and Schilde, 1978a; 5. Hayes, 1985; 6. Kowalski *et al.*, 1986; 7. Eigenberg, 1991a; 8. Clemens *et al.*, 1987; 9. Harris, 1965; 10. Thyssen *et al.*, 1977; 11. Abou-Donia *et al.*, 1979b; 12. Hansen *et al.*, 1982; 13. Sheets *et al.*, 1991; 14. Thyssen and Schilde, 1978b; 15. Sheets, 1991b.

[b] Estimated assuming a respiratory rate of 0.24 and 0.17 m^3/kg/6 hrs for a rat and hen, respectively.

[c] ChE = cholinesterase. Inhibition is expressed as percent of control activity.

[d] Developmental toxicity study: Only maternal effects observed after the first few days of exposure were included.

[e] Reproductive toxicity study

* Acceptable study based on FIFRA guidelines

were observed in a 2-generation rat reproductive toxicity study including a reduction in fertility, birth and viability indices, an increase in gestation length, reduced pup weights, cannibalism of pups, and discolored pup livers. Although the reproductive toxicity of DEF was only examined with oral exposure, it was assumed that these effects would occur with exposure by any route. Dermal irritation was also observed with dermal exposure.

Significantly lower NOELs for DEF were observed with two oral subchronic studies. In an acceptable reproductive toxicity study, a NOEL of 4 ppm (0.4 mg/kg/day) was established based on brain ChE inhibition (71% of control activity) in adult female rats at 32 ppm (2.4 mg/kg/day) (Eigenberg, 1991a). There were gender-related differences in ChE activity in this study which were most pronounced at the terminal sacrifice. However, gender-related differences in brain ChE activity were not observed in the 2-year feeding study in rats where the reduction in brain ChE inhibition at the LOEL, 320 ppm (19 mg/kg/day), was similar in both sexes (M:40%; F:32% of controls) (Christenson, 1992). In fact, the NOEL for brain ChE inhibition in the 2-year feeding study (M:1.8 mg/kg/day; F: 2.3 mg/kg/day) was comparable to the NOEL for brain ChE inhibition in males in a rat reproductive toxicity study (2.2 mg/kg/day). An increased sensitivity in pregnant females also does not appear to be a likely explanation because the maternal NOEL for brain ChE inhibition in the rat developmental toxicity study was 7 mg/kg/day (Kowalski *et al.*, 1986). A more likely explanation for these gender-related differences in the reproduction study was the higher compound consumption in females during lactation. During lactation, the average compound consumption for females in both generations was approximately twice as high as their consumption during premating and gestation (0.7, 5.5, and 39.2 mg/kg/day at 4, 32, and 260 ppm, respectively). Because of the route of exposure and the uncertainty regarding the impact of the increased compound consumption in the females during lactation on the brain ChE activity, this study was not selected as the definitive study for evaluating seasonal occupational exposure to DEF.

A NOEL of 0.1 mg/kg/day was reported in a neurotoxicity study in hens in which mild ataxia was observed at 0.5 mg/kg/day when DEF was administered in capsules for 90 days (Abou-Donia *et al.*, 1979b). It is unclear if the ataxia with oral exposure was due to DEF or nBM which can be formed in the gastrointestinal tract of hens from the hydrolysis of DEF (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). There was limited histological evidence of OPIDN with oral exposure even at high doses. By contrast, similar doses of DEF administered by the dermal route produced clear evidence of OPIDN. Hens as 20 mg/kg/day and higher had severe ataxia and paralysis and died within the first few weeks from late effects attributed to nBM. The limited histological evidence of OPIDN with oral exposure, suggests that the ataxia and paralysis is due to nBM rather than DEF. Abou-Donia and others have reported that nBM causes incoordination, muscle weakness, paralysis, CNS depression and cyanosis in rats and/or hens (Fairchild and Stokinger, 1958; Abou-Donia *et al.*, 1979a). If sufficient amounts of DEF are degrading to nBM with oral exposure to kill hens at 20 mg/kg/day and higher, then less DEF should be available to produce OPIDN. Less weight was also given to the Abou-Donia *et al.* (1979b) study because of the lack of detail in the published report regarding the incidence and duration of clinical signs, body weight changes, and histopathological lesions. The study also did not meet FIFRA guidelines with respect to the number and age of animals and analysis of the test article. It is difficult to interpret the findings of this study without more information, especially considering the NOEL is an order of magnitude lower than the NOELs for any other subchronic neurotoxicity study in hens, including one which met FIFRA guidelines (Sheets, 1991b).

A. HAZARD IDENTIFICATION (cont.)

In selecting a definitive study for evaluating occupational exposure to DEF, preference was given to subchronic dermal studies. The lowest dermal LOELs were observed in a 3-week study in rabbits and a 13-week study in hens. Both of these studies were acceptable based on FIFRA guidelines. In rabbits, muscle fasciculations, reduced brain ChE activity (85-86% of controls), and microscopic lesions in the skin (acanthosis and hyperkeratosis) were observed in both sexes at 11 mg/kg/day (Sheets *et al.*, 1991). In the neurotoxicity study, axonal degeneration was observed in hens at 11 mg/kg/day (Sheets, 1991b). Although both of these studies were acceptable, there was a high background rate for axonal degeneration in the hen study due to the age of the birds which confounded the interpretation of the histological findings. Therefore, the subchronic dermal toxicity study in rabbits was selected as the definitive study for evaluating the seasonal occupational exposure with a critical NOEL of 2.0 mg/kg/day based on muscle fasciculations, brain ChE inhibition (85-86% of controls) and microscopic skin lesions. After correcting for dermal absorption (47.5%), the adjusted NOEL was 0.95 mg/kg/day.

Pre- and Post-natal Sensitivity

Developmental toxicity studies in rats and rabbits and reproductive toxicity studies in rats were considered in assessing the potential for higher sensitivity in infants and children than adults. Two developmental toxicity studies were conducted in which DEF was administered by oral gavage, one in rats and the other in rabbits (Kowalski *et al.*, 1986; Clemens *et al.*, 1987). Both studies met FIFRA guidelines. No treatment-related increases in embryotoxicity, fetal malformations or variations were observed in rats and rabbits. Maternal effects included brain ChE inhibition and reduced body weight gain. In rats, the maternal brain ChE activity was reduced (54% of controls) at 28 mg/kg/day on day 20 of gestation; however, fetal brain ChE activity was unaffected. Reductions in the average maternal body weight gain were observed in rats and rabbits at 28 and 9 mg/kg/day, respectively, without corresponding reductions in fetal body weights. These findings in rats and rabbits suggest there is no increased prenatal sensitivity to DEF.

One reproductive toxicity study was available in which DEF was administered in the feed to rats (Eigenberg, 1991a). The study met FIFRA guidelines. Several reproductive effects were seen in this study. The reproductive effects included reductions in the fertility, birth, and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, brain ChE inhibition and gross pathological lesions in pups. The reproductive NOEL was 32 ppm (3.0 mg/kg/day). Other non-reproductive effects in the adults included brain ChE inhibition and body weight reductions. A reduction in mean brain ChE activity was observed in females (71% of controls) at 32 ppm on day 21 of lactation. No reduction in brain ChE activity was observed in the 21-day-old pups at 32 ppm. At 260 ppm, the reduction in the mean brain ChE activity was significantly greater in adult females (F_0&F_1:19% of controls) than the 21-day-old pups (F_{2a}:85% of controls). The mean body weight reductions in the adult females (24%) was similar to the reductions in the 21-day-old pups (25%) at 260 ppm. Based on these findings in rats, there does not appear to be any increased postnatal sensitivity to DEF.

Chronic Toxicity

In all of the available chronic toxicity/oncogenicity studies, DEF was administered to animals in their feed. Several adverse effects were seen in these chronic toxicity/ oncogenicity studies (Table 17). Reduced weight gain was observed in two rat chronic toxicity studies at 100

Table 17. Chronic Adverse Effects of DEF and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL mg/kg/day	LOEL mg/kg/day	Ref.[a]
Mouse	Diet, 90 weeks	Vacuolar degeneration in small intestine, hematopoiesis in spleen, hematological changes (F), brain ChE[b] inhibition (M:87%)	1.5	8.4	1*
Rat	Diet, 2 years	Liver cytoplasmic vacuolation, reduced weight gain, brain ChE inhibition (F:69%)	1.25	5.0	2
Rat	**Diet, 2 years**	**Hyperplasia and vacuolar degeneration in small intestine, hematological changes**	**0.2**	**1.8**	**3***
Dog	Diet, 1 year	Hematological changes (F)	0.4	2.0	4*

[a] References: 1. Hayes, 1989; 2. Root *et al.*, 1967; 3. Christenson, 1992; 4. Christenson, 1991.
[b] ChE = cholinesterase. Inhibition expressed as percent of control activity.
* Acceptable study based on FIFRA guidelines

ppm (5 mg/kg/day) and 320 ppm (M: 16.8 mg/kg/day; F: 21.1 mg/kg/day) (Root *et al.*, 1967;Christenson, 1992). The mean brain ChE activity was significantly reduced in males of the mouse oncogenicity study at 50 ppm (8.4 mg/kg/day - 87% of controls), and in two rat chronic feeding study at 100 ppm (F: 5 mg/kg/day - 69% of controls) and 320 ppm (M: 16.8 mg/kg/day - 40% of controls; F: 21.1 mg/kg/day - 32% of controls) (Hayes, 1989; Root *et al.*, 1967; Christenson, 1992). The mean brain ChE activity was also significantly reduced (91% of controls) in male mice at 10 ppm (1.5 mg/kg/day); however, it was not considered toxicologically significant because no cholinergic signs were observed at 50 ppm and only mild signs (loose stools and perianal stains) were observed at 250 ppm. Hematological changes (reduced RBCs, hemoglobin and hematocrits) were seen in female mice at 50 ppm (11.3 mg/kg/day), in rats of both sexes at 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively), and in female dogs at 64 ppm (2.0 mg/kg/day) (Hayes, 1989; Christenson, 1991; Christenson, 1992). Transient hypothermia was also observed in one rat chronic toxicity study at 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively) (Christenson, 1992).

There were dose-related increases in microscopic lesions in several studies. In a mouse oncogenicity study, an increase in non-neoplastic lesions in the gastrointestinal tract (small intestine vacuolar degeneration, dilated/distended small intestine and cecum, and rectal necrosis/ulceration), liver (hypertrophy), adrenal glands (degeneration/ pigmentation), and spleen (hematopoiesis) was observed at 250 ppm (M: 48.1 mg/kg/day; F: 63.1 mg/kg/day) (Hayes, 1989). The incidence of small intestine vacuolar degeneration and spleen hematopoiesis was also significantly higher in mice at 50 ppm (M: 8.4 mg/kg/day; F: 11.3 mg/kg/day). Increases in several pre-neoplastic lesions in the small intestine of both sexes (mucosal hyperplasia and focal atypia) and the lungs of females (epithelialization and focal hyperplasia) were also observed at 250 ppm (M: 48.1 mg/kg/day; F: 63.1 mg/kg/day). Liver

cytoplasmic vacuolation was observed in a rat chronic feeding study at 100 ppm (5 mg/kg/day) (Root *et al.*, 1967). Numerous ocular effects were seen in another rat chronic feeding at 320 ppm (M: 16.8 mg/kg/day; F: 21.1 mg/kg/day) including corneal opacity, lens opacity, cataracts, corneal neovascularization, iritis, uveitis, bilateral flat ERG responses, bilateral retinal atrophy, and optical nerve atrophy (Christenson, 1992). In addition, increased adrenal weights and adrenal vacuolar degeneration were observed at 320 ppm. Vacuolar degeneration of the small intestine was seen in animals exposed for one or two years at both 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively). Mucosal hyperplasia was also observed at 40 and 320 ppm, but only in animals exposed for two years.

The most sensitive endpoint with chronic oral exposure to DEF appears to be the hematological effects. There is evidence in hens that these hematological effects may be due to nBM which inhibits glucose-6-phosphate dehydrogenase (Abdo *et al.*, 1983b). In previous experiments, these investigators isolated nBM in the plasma and excreta of hens and proposed that it is the product of hydrolysis of DEF in the gut (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators also found nBM in the plasma of hens administered DEF dermally, although the concentration was an order of magnitude lower than when the same dose was given orally (Abou-Donia *et al.*, 1979a). Although the hematological changes may be more prevalent with the oral route of exposure, they do not appear to be unique to this route of exposure. nBM is an anticipated metabolite of DEF through its normal metabolism in the liver of animals. Also, DEF readily degrades to nBM in the environment. Slight reductions in erythrocyte counts, hematocrits, and hemoglobin values were also seen in a 90-day inhalation study in rats (Pauluhn, 1992). The significance of these hematological effects in assessing the long-term human health risks from occupational exposure to DEF is uncertain because exposure is clearly seasonal and these hematological effects appear to be reversible. In the chronic feeding study in rats, some of the hematological values began to return to normal even with continued exposure (Christenson, 1992). In fact, the RBC count, hemoglobin and hematocrit values were higher in the high-dose animals than controls by the end of the 2-year exposure period.

The relevance of the histological lesions in the adrenal gland, small intestine, liver and spleen of animals after chronic oral exposure to DEF is also uncertain because similar histological changes were not observed in a 3-week dermal toxicity study in rabbits where these tissues were also examined microscopically (Sheets *et al.*, 1991). ERG's were not taken in the rabbit study, so it is uncertain if these lesions were absent or simply not detected. However, the LOEL of 11 mg/kg/day in the 3-week rabbit study was similar to the LOEL of 14.3 mg/kg/day in a 13-week inhalation study in rats at which lesions in the adrenal gland and eyes were observed (Pauluhn, 1992). The lack of concordance in the histological findings between the subchronic and chronic studies could be due to the difference in either the duration or the route of exposure or possibly species differences. Some of these histological lesions, like the vacuolar degeneration and mucosal hyperplasia in the small intestine, could be due to local irritation, from DEF or nBM, and may only be relevant for the oral route of exposure. Consequently, these lesions were not considered relevant to occupational exposure because of the route of exposure. Other effects in the chronic studies, which also appeared to be reversible, such as the hematological changes and brain ChE inhibition, were not of a concern for long term exposure because occupational exposure is limited to a few months of the year.

Chronic dietary exposure to DEF may be expected due to year-round consumption of cottonseed oil, meal or milk and meat containing secondary residues from livestock consuming

cottonseed products. The lowest chronic NOEL was observed in an acceptable 2-year rat study based on the histological changes in the small intestine and hematological changes (Christenson, 1992). The NOEL of 0.2 mg/kg/day from the 2-year rat study was similar to the lowest subchronic oral NOEL of 0.1 mg/kg/day (Abou-Donia *et al.*, 1979b) based on mild ataxia in hens. However, this subchronic study had several deficiencies including inadequate number of animals, no analysis of test article, inadequate summary of clinical signs, body weights and histopathological findings, and the age of animals. The 2-year rat study was selected as the definitive study for evaluating the chronic dietary exposure to DEF. After correcting for oral absorption (70%), the adjusted critical NOEL was 0.14 mg/kg/day.

Weight of Evidence for Oncogenicity

There was no evidence of oncogenicity in a rat study where DEF was administered in the feed for 2 years (Christenson, 1992). However, an increase in adenocarcinomas of the small intestine (both sexes), liver hemangiosarcomas (males only), and alveolar/bronchiolar adenomas (females only) was seen in mice fed DEF for 90 weeks (Tables 9 and 10) (Hayes, 1989). The adenocarcinomas were often associated with vacuolar degeneration, mucosal hyperplasia and/or focal atypia of the small intestine. The liver hemangiosarcomas were often associated with hemorrhage and necrosis. The increase in alveolar/bronchiolar adenomas was often associated with epithelization and focal hyperplasia. Both oncogenicity studies met FIFRA guidelines.

In the mouse oncogenicity study, there was a high incidence of marked anemia, fluid-filled or dilated intestines, degenerative lesions in the adrenal gland and gastrointestinal tract, and increased non-oncogenic mortality (females) at 250 ppm suggesting that the maximum tolerated dose (MTD) was exceeded. This excessive toxicity might be due to saturation of metabolic pathways which could lead to an increase in a tumor incidence through the accumulation of or formation of more reactive metabolites (Carr and Kolbye, 1991). Increased cell proliferation due to cytotoxicity can result in the promotion of endogenous DNA damage by decreasing the time available to repair DNA damage (Swenberg, 1995). Other non-genotoxic mechanisms could be responsible for the increase in tumor incidences including immunosuppression or an alteration in hormone levels (MacDonald *et al.*, 1994). Theoretically, a biological threshold could exist for the oncogenic response if there is evidence that the metabolic pathways are saturated or the endocrine or immune systems are dysfunctional at doses where there is an increased tumor incidence.

There was no evidence of genotoxicity in the four available studies for DEF (an Ames assay, an *in vitro* chromosomal aberrations assay, an *in vitro* sister chromatid exchange assay, and an unscheduled DNA synthesis assay). All of these genotoxicity studies met FIFRA guidelines, except the sister chromatid exchange assay which was a published report.

Quantitative Assessment of Oncogenic Effects

Although the increase in small intestine adenocarcinomas and alveolar/ bronchiolar adenomas only occurred on at the highest dose level where there was evidence of excessive toxicity, there was insufficient data for DEF to indicate whether any threshold mechanisms might be responsible for the oncogenic response. Moreover, multiple tumor sites were involved, one of which is a rare tumor type (small intestine adenocarcinoma) with a reported historical control range for this laboratory of 0% in both sexes. Consequently, it was assumed there was no

threshold and the potential oncogenic risk to humans was evaluated using a linear, low dose extrapolation model to estimate potency.

It was not possible to accurately estimate the oncogenic potency (Q_1) or upper bound (Q_1*) on the slope for small intestine adenocarcinomas and alveolar/bronchiolar adenomas because the slope estimate (Q_1) is zero when the tumor incidence is only increased at the high dose. Therefore, the incidence of liver hemangiosarcomas in male mice was used to calculate the oncogenic potency of DEF. Due to the reduced survival of mice at the highest dose tested, 250 ppm, the oncogenic potency of DEF was estimated using the multistage-Weibull time-to-tumor model, MULTI-WEIB. The dosages for male mice (0, 1.5, 8.4 or 48.1 mg/kg/day) were converted to human equivalent dosages multiplying by an interspecies scaling factor of body weight to the 3/4 power [$(BWt_A/BWt_H)^{0.25}$ = (0.030 kg/70 kg)$^{0.25}$ = 0.144]. The estimated oncogenic potency ranged from 3.3 x 10^{-2} (maximum likelihood estimate or MLE) to 5.9 x 10^{-2} (95% upper bound or 95% UB) (mg/kg/day)$^{-1}$.

n-Butyl Mercaptan

Only limited animal toxicity data were available for nBM. Effects observed in a battery of acute toxicity tests (intraperitoneal LD50, oral LD50, inhalation LD50, ocular irritation) were indicative of CNS depression including incoordination, muscular weakness, paralysis, lethargy, sedation, respiratory depression, cyanosis, and coma (Fairchild and Stokinger, 1958). Other effects included restlessness, increased respiration, diarrhea (oral exposure), sneezing (inhalation exposure), and ocular irritation. Liver damage (lymphatic infiltration and necrotic foci with small hemorrhages) and kidney damage (cloudy swelling of the tubules and hyaline casts in the lumina) were observed with all routes of exposure. With inhalation exposure, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage were seen. There was insufficient information available in the published report by Fairchild and Stokinger (1958) to establish a NOEL by any of the routes tested. An inhalation developmental toxicity study was available in which mice and rats were exposed to vapors of nBM for 6 hrs/day on gestation days 6-16 and 6-19, respectively (Thomas *et al.*, 1987). No maternal or developmental effects were seen in rats. The NOEL for the maternal and developmental effects in mice was 10 ppm (17 mg/kg/day) based on increased mortalities, reduced body weight gain, unkempt appearance, lethargy, red/brown perianal stains, increased post-implantation losses, and fetal malformations.

As mentioned earlier, the “late acute” effects seen in hens with oral exposure were attributed to nBM which is probably formed in the gut from the hydrolysis of DEF (Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984). These investigators tested this theory by administering nBM to hens and found they developed signs similar to those described as late acute effects (malaise, leg or general weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors, loss of breath, and dark and droopy comb just prior to death), except the onset of signs was earlier (6-12 hrs after administration). Hens administered nBM did not respond to atropine therapy, did not have any inhibition of brain or plasma ChE activity, and did not develop degenerative changes in peripheral nerves. A NOEL of 100 mg/kg was observed based on clinical signs in hens administered nBM. Abdo *et al*. (1983b) observed erythrocyte deformation and lysis in hens 24-48 hrs after administering nBM at 500 mg/kg. Methemoglobin levels were elevated while erythrocyte counts, hematocrit, hemoglobin levels and G-6-PD activity were reduced. Because the time course of the hematological changes and the late acute effects were similar, the investigators proposed that the inhibition of G-6-PD was responsible for the

hematological changes. G-6-PD is involved in the regeneration of NADPH which in turn is needed for the reduction of glutathione. A reduction in glutathione levels could lead to the formation of methemoglobin and Heinz bodies, coagulation of surface proteins on erythrocytes, leading to deformation and eventual cell lysis. Since only one dose was administered in this study, a NOEL was not established for the hematological changes.

Residents of agricultural communities in cotton-growing regions have complained of eye and throat irritation, rhinitis, wheezing, coughing, shortness of breath, nausea and diarrhea during the time of cotton defoliation with DEF (Maddy and Peoples, 1977; Scarborough, 1989). Based on the acute effects seen in animals exposed to nBM, it appears that nBM may be responsible for the ocular and respiratory irritation. Some of these complaints may also be due to the strong skunk-like odor. The an odor threshold of nBM in humans between 0.01 and 1.0 ppb (Santodonato *et al.*, 1985). Offensive odors may trigger symptoms in humans, such as nausea and headache, by indirect physiologic mechanisms including exacerbating an underlying medical condition, innate odor aversion, odor-related aversive conditioning, stress-induced illness, and possible innate pheromonal reaction (Shusterman, 1992). Ames and Stratton (1991) analyzed health effects reported by residents living near a potato field that had been treated with ethoprop which breaks down to n-propyl mercaptan. They found that symptoms more closely correlated with odor perception than with distance from the potato field.

B. EXPOSURE ASSESSMENT

Occupational Exposure

Exposure estimates for handlers involved in the aerial or ground application of DEF to cotton are summarized in Table 18. The total daily exposure by the dermal and inhalation routes are the geometric means from the data presented in Tables 2 and 3 of the Exposure Assessment Document for DEF which comes primarily from a study conducted by Eberhart (1993), except for flaggers (Formoli and Wang, 1995). A weighted average of the exposure data from both the Eberhart (1993) and Peoples *et al.* (1981) studies were used for flaggers. The exposure data for mixer/loaders and pilots from the Peoples *et al.* (1981) study was not used because of the inconsistent use of protective clothing. The dermal exposure represented more than 95% of the total daily exposure for handlers. To address the risk for severe dermal irritation from the DEF formulation, the average hand exposure (µg/person/day) was divided by the concentration of DEF in the formulation (70%) and the surface area of the hand (990 cm^2). The hand exposure ranged from 0.20 µg formulation/cm^2 for ground applicators to 5.48 µg formulation/cm^2 for pilots. The Absorbed Daily Dosage (ADD) was the geometric mean of the total daily exposure by the dermal and inhalation routes assuming an average worker weighs 75.9 kg, dermal absorption is 47.5%, and respiratory uptake is 50% with DEF as a vapor during occupational exposure. The ADD was greatest for mixer/loaders involved in ground application of DEF (54.3 µg/kg/day). The ground applicators had the lowest exposure (4.4 µg/kg/day). The Seasonal Average Daily Dosage (SADD) was calculated from the ADD assuming 21 days of exposure in a 45-day use season for cotton. The SADDs ranged from 2.1 to 25.3 µg/kg/day. Because occupational exposure was limited to a 45-day use season per year, a chronic exposure dosage (i.e., Annual Average Daily Dosage) was not calculated. The Lifetime Average Daily Dosage (LADD) is used to calculate the oncogenic risk for workers. The LADDs for pesticide workers ranged from 0.2 to 1.8 µg/kg/day for handlers.

B. EXPOSURE ASSESSMENT (cont.)

Table 18. Estimated Exposure Dosages in Pesticide Workers for DEF Use on Cotton[a]

Job Categories	Hand Exposure[b] µg DEF EC/cm^2	ADD[c]	SADD[d] µg/kg/day	LADD[e]
Aerial Application				
Mixer/Loader	1.84	28.1	13.1	0.9
Pilot	5.48	33.2	15.5	1.1
Flagger	0.57	23.7	11.1	0.8
Ground Application				
Mixer/Loader	5.01	54.3	25.3	1.8
Applicator	0.23	4.4	2.1	0.1
Harvesters				
Picker Operator	------	94.3	44.0	3.1
Module Builder Operator	------	29.8	13.9	1.0
Raker	------	56.0	26.1	1.8
Tramper	------	121.8	56.8	4.0

[a] Exposure estimates for handlers are from Eberhart (1993), except for flaggers which is the weighted average of exposure estimates from both Peoples *et al.* (1981) and Eberhart (1993). A 7-hour workday was assumed for aerial application and 8-hour workday for ground application. Exposure estimates for harvesters were calculated using dermal transfer factors and the average residue level on cotton bolls at 7 days after application derived from a study conducted by Eberhart and Ellisor (1993). Inhalation exposure was assumed to be negligible for harvesters.

[b] The hand exposure (µg/person/day) for handlers was converted to µg of DEF emulsifiable concentrated (EC) per cm^2 by dividing by the concentration of DEF in the formulation (70%) and the surface area of the hand (990 cm^2).

[c] ADD = Absorbed Daily Dosage assuming 47.5% dermal absorption, 50% respiratory uptake of DEF as a vapor with occupational exposure, and 75.9 kg body weight. The value represents the geometric mean for handlers and the arithmetic mean for harvesters based on the distribution of the data.

[d] SADD = Seasonal Average Daily Dosage assuming workers are exposed 21 days in a 45-day season

[e] LADD = Lifetime Average Daily Dosage assuming an exposure over 40 years of a 70-year lifespan.

The exposure estimates for workers harvesting cotton treated with DEF are also summarized in Table 18. The exposure estimates were derived using dermal transfer factors derived from a study conducted by Eberhart and Ellisor (1993) in which cotton treated with DEF was harvested 15 to 20 days after application. The arithmetic mean of the dermal exposure was used in calculating the dermal transfer factors. The estimated dermal transfer factors ranged from 2832 g/hr for rakers to 6157 g/hr for trampers. The dermal transfer factors were then used to estimate exposure if the cotton had been harvested at the minimum pre-harvest interval of 7 days after application using residue data that was also collected in this same study. Predicted residue levels were estimated from observed levels using log-linear regression analysis. The predicted residues for day 7 with aerial and ground application were 0.47 and 0.32 µg/g cotton boll, respectively. The average of these two residue levels, 0.395 µg/g, was multiplied by the dermal transfer factor to estimate dermal exposure for the specific job category. Hand exposure to the DEF formulation was not calculated for cotton harvesters

because it was assumed that the inert ingredients were volatile and had dissipated by the time of harvesting. Consequently, these workers were exposed primarily to technical grade DEF which only caused mild dermal irritation. The same assumptions made for handlers regarding dermal absorption, body weight, and days worked per season were used for cotton harvesters. The ADDs ranged from 29.8 µg/kg/day for module builder operators to 121.8 µg/kg/day for trampers. The SADDs ranged from 13.9 to 56.8 µg/kg/day. The LADDs for harvesters ranged from 1.0 to 4.0 µg/kg/day.

Dietary Exposure

DPR evaluates the risk of exposure to an active ingredient in the diet using two processes: (1) use of residue levels detected in foods to evaluate the risk from total exposure, and (2) use of tolerance levels to evaluate the risk from exposure to individual commodities (see Tolerance Assessment section). For the evaluation of risk to detected residue levels, the total exposure in the diet is determined for all label-approved raw agricultural commodities, processed forms, and animal products (meat and milk) that have established U.S. EPA tolerances. Tolerances may be established for the parent compound and associated metabolites. DPR considers these metabolites and other degradation products that may be of toxicological concern in the dietary assessment.

Residue Data

The sources of residue data for dietary exposure assessment include DPR and federal monitoring programs, field trials, and survey studies. Residue data obtained from the monitoring programs are preferred because they represent a realistic estimate of potential exposure. In the absence of data, surrogate data from the same crop group as defined by U.S. EPA or theoretical residues equal to U.S. EPA tolerances. Residue levels that exceed established tolerances (over-tolerance) are not utilized in the dietary exposure assessment because over-tolerance incidents are investigated by the DPR Pesticide Enforcement Branch and are relatively infrequent. DPR evaluates the potential risk from consuming commodities with residues over tolerance levels using an expedited acute risk assessment.

DPR has two major sampling programs: priority pesticide and marketplace surveillance. The priority pesticide program focuses on pesticides of health concern as determined by DPR Enforcement and Medical Toxicology Branches. Samples are collected from fields known to have been treated with the specific pesticides. For the marketplace surveillance program, samples are collected at the wholesale and retail outlets, and at the point of entry for imported foods. The sampling strategies for both priority pesticide and marketplace surveillance are similar and are weighted toward such factors as pattern of pesticide use; relative number and volume of pesticides typically used to produce a commodity; relative dietary importance of the commodity; past monitoring results; and extent of local pesticide use.

The U.S. Food and Drug Administration (FDA) has three programs for determining examining residues in food: (1) regulatory monitoring, (2) total diet study, and (3) incidence/level monitoring. For the surveillance monitoring, surveillance samples are collected from individual lots of domestic and imported foods at the source of production or at the wholesale level. In contrast to the regulatory monitoring program, the total diet study monitors residue levels in the form that a commodity is commonly eaten or found in a prepared meal. The incidence /level

monitoring program is designed to address specific concerns about pesticide residues in particular foods.

The U.S. Department of Agriculture (USDA) is responsible for the Pesticide Data Program (PDP), a nationwide cooperative monitoring program. The PDP is designed to collect objective, comprehensive pesticide residue data for risk assessments. Several states, including California, collect samples at produce markets and chain store distribution centers close to the consumer level. The pesticide and produce combinations are selected based on the toxicity of the pesticide as well as the need for residue data to determine exposure. In addition, USDA is responsible for the National Residue Program which provides data on potential pesticide residues in meat and poultry. These residues in farm animals can occur from direct application or consumption of commodities or by-products in their feed.

Primary Residues

DPR added DEF to the multi-residue screen in its marketplace surveillance and priority pesticide programs for 1991. No DEF residues in cottonseed products have been detected since its inclusion in the multi-residue screen, but this is not surprising since neither cottonseed meal nor oil products are sampled by DPR (DPR, 1992-1993). The FDA has routinely analyzed cottonseed, refined cottonseed oil, milk, and meat for DEF residues since 1985. However, the minimum detection limits (MDLs) are unknown due to insufficient residue/matrix combination information. The MDL for DEF will likely be in the same range as the other related organophosphate pesticides monitored by the FDA. The FDA MDL range for related organophosphate pesticides varies from 0.01 to 0.1 ppm for the assorted raw agricultural commodities and their derivatives (FDA, 1995). There were no detected residues of DEF found in the 8 cottonseed product samples tested during the fiscal years 1990-1995 surveillance period in the FDA monitoring programs. The USDA National Residue Program and the Pesticide Data Program do not monitor for DEF residues.

There were 36 field studies of cottonseed samples submitted to DPR by the registrants (Chemagro Corp, 1965a & 1969). Fifty percent of these studies were not suitable for consideration in the dietary exposure analysis because the field study application rates and/or the pre-harvest intervals did not approximate those on the current labels for DEF. The residue values for cottonseed were generated from 18 registrant studies where DEF was applied at 30-42 oz/acre and samples were collected at 7 days post application (Chemagro, 1965a&b, 1969). The maximum and mean residue level of these 18 samples was 2.60 and 0.87 ppm, respectively. The MDL for all of these studies was 0.1 ppm.

Anticipated residue levels of DEF in processed cottonseed products were derived by multiplying the level in whole cottonseed by various processing factors (Table 19). The processing factors for refined cottonseed hulls, meal, oil, and gin trash were 1.24, 0.04, 0.29, and 6.76, respectively, based on some limited residue data submitted by the registrant for DEF in processed cottonseed (Chemagro Corp., 1965b & 1969). All cottonseed oil prepared for human consumption undergoes an additional processing step (deodorization) to remove aromatics and low boiling point constituents. Approximately 99% of the DEF was removed from cottonseed oil in a simulation of this process in which the oil was steam stripped (Thornton, 1968). However, no residue data were submitted for deodorized cottonseed oil after going through the normal deodorization process; therefore, the residue levels in cottonseed oil were not adjusted for this additional processing step.

B. EXPOSURE ASSESSMENT (cont.)

Table 19. Processing Factors and Anticipated Residue Levels of DEF in Processed Cottonseed

Product	Processing Factor[a]	Anticipated DEF Residues Maximum (ppm)	Anticipated DEF Residues Mean (ppm)
Whole Cottonseed[b]	----	2.60	0.87
Cottonseed Hulls	1.24	3.22	1.08
Cottonseed Meal	0.04	0.10	0.03
Refined Cottonseed Oil	0.29	0.75	0.25
Cotton Gin Trash	6.76	17.58	5.88

[a] The processing factors were based on DEF residues in a few samples of processed cottonseed (Chemagro Corp., 1965b & 1969).

[b] The DEF residue levels in whole cottonseed were based on field studies conducted by registrant (Chemagro Corp., 1965a).

Secondary Residues

The registrant provided residue studies for cattle, goats and poultry (Chemagro Corp., 1968a&b; Sahali, 1991; Hall, 1991). DEF was administered to cattle at 84.5 mg/1000 lb/day (0.19 mg/kg/day) in capsules for 28 days (Chemagro Corp., 1968a&b). This dose level was theoretically equivalent to that which the animal would receive if it consumed 3% of its body weight in feed that was 50% cottonseed hulls containing DEF at 12.4 ppm. The theoretical residue level of the hulls in the animal feed was based on the highest residue level detected in whole cottonseed, 9.06 ppm, in the field studies submitted by the registrant and adjusting for the higher concentration of DEF in the hull. However, the highest residue level in the whole cottonseed occurred when DEF was applied at twice the maximum application rate. To obtain more realistic DEF residue levels in cattle tissues and milk, the relationship between the administered dose and the tissue level was assumed to be proportional and the tissue levels were adjusted by a distribution factor (Table 20). The distribution factor is simply the ratio of the residue level in the tissue to the level in the diet. The amount of processed cottonseed products in feed was assumed to be 10, 25, 20, and 30% for meal, seeds, hulls, and gin trash, respectively, based on the U.S. EPA guidelines for residue studies (U.S. EPA, 1994). The maximum theoretical residue in animal feed from the various cottonseed products was estimated to be 6.58 ppm. The mean theoretical residue in animal feed was 2.20 ppm.

Two lactating goats were administered DEF in capsules at 0.82 and 0.85 mg/kg/day on 3 consecutive days (Sahali, 1991). These dosages were theoretically equivalent to residue levels of 109 and 113 ppm, respectively, for whole cottonseed if 25% of the feed is from cottonseed products and goats consume 3% of their bodyweight in feed daily. These theoretical residue levels are approximately 25 times the tolerance level for whole cottonseed. As with cattle, more realistic DEF residue levels in goat tissues and milk were derived by assuming the tissue levels were proportional to dose and adjusting by a distribution factor (Table 21). Although the total radioactivity in tissues was measured, only the residues of the parent compound were used in the estimation of dietary exposure. DEF represented 36, 5, and

B. EXPOSURE ASSESSMENT (cont.)

Table 20. Distribution Factors and Anticipated DEF Residues in Cattle Tissues and Milk

Tissue	Distribution Factor[a] (%)	Anticipated DEF Residues[b]	
		Maximum (ppm)	Mean (ppm)
Brain	0.37	0.02[c]	0.008
Heart	0.51	0.02[c]	0.011
Liver	0.44	0.02[c]	0.010
Kidney	0.55	0.02[c]	0.012
Muscle	0.25	0.016	0.006
Fat	0.32	0.02[c]	0.007
Milk	0.05	0.002[c,d]	0.0008[d]

[a] The distribution factors were based on the residue levels found in cattle tissues after administering DEF at 84.5 mg/1000 lb/day (0.19 mg/kg/day) for 28 days (Chemagro Corp., 1968a&b). The distribution factor is the ratio of the residue level in the tissue to the level in the diet.

[b] The maximum and mean theoretical residue level in feed for beef cattle was estimated to be 6.59 and 2.20 ppm, respectively, assuming the feed contained meal, seeds, hulls, and gin trash at 10, 25, 20 and 30%, respectively (U.S. EPA, 1994). The maximum or mean theoretical residue levels in tissues were estimated by multiplying the distribution factor by the maximum or mean theoretical residue level in the feed (e.g., the mean residue in cattle brain = 0.37% x 2.20 ppm = 0.008 ppm).

[c] Based on the maximum theoretical residue in feed of 6.59 ppm, the maximum anticipated residue in brain, heart, liver, kidney, fat, and milk exceeded the tolerance; therefore, the residue levels were set at the tolerance.

[d] The maximum and mean theoretical residue level in feed for dairy cattle was estimated to be 4.66 and 1.56 ppm, respectively, assuming the feed contained meal, seeds, hulls, and gin trash at 15, 25, 15 and 20%, respectively (U.S. EPA, 1994).

<1% of the total radioactive residues in fat, milk, and other tissues, respectively. The other radioactive components in the tissues did not match the reference standards for various known degradation products of DEF including nBM, S,S-dibutyl phosphorodithioate, and S-butyl phosphorothioate. The majority of the radioactivity was found in the protein and fatty acid fraction of the tissues. The percentage of cottonseed products in the feed of goats was assumed to be identical to cattle (i.e., 10% meal, 25% seeds, 20% hulls, and 30% gin trash).

The distribution factors for goat tissue and milk were significantly lower than those for cattle. Most likely this is due to the shorter exposure period (3 days vs. 28 days) in the goat study; however, differences in methodology and metabolism may also be involved. Since species differences in metabolism are likely and could not be eliminated as a possible cause, different distribution factors were used for goat and cattle tissues. No residue studies were available for sheep tissues; therefore, the distribution factors for goat were used for sheep.

Currently, there are no tolerances for poultry or eggs, so the residue levels from the metabolism study with laying hens was not incorporated into the dietary exposure (Hall, 1991).

B. EXPOSURE ASSESSMENT (cont.)

Table 21. Distribution Factors and Anticipated DEF Residues in Goat Tissues and Milk

Tissue	Distribution Factor[a] (%)	Anticipated DEF Residues[b]	
		Maximum (ppm)	Mean (ppm)
Liver	0.126	0.0083	0.0028
Kidney	0.014	0.0009	0.0003
Fat	0.245	0.0161	0.0054
Muscle	0.002	0.00013	0.00004
Milk	0.022	0.0010[c]	0.0003[c]

[a] The distribution factors were based on the residue levels found in goat tissues after administering DEF at 0.82-0.85 mg/kg/day for 3 days (Sahali, 1991). The distribution factor is the ratio of the residue level in the tissue to the level in the diet.

[b] The maximum and mean theoretical residue in feed for goat was estimated to be 6.58 and 2.20 ppm, respectively, assuming the feed is similar to beef cattle which contained meal, seeds, hulls, and gin trash at 10, 25, 20, and 30%, respectively. The maximum or mean theoretical residue levels in tissues were estimated by multiplying the distribution factor by the maximum or mean theoretical residue level in the feed (e.g., the mean residue in goat liver = 0.126% x 2.20 ppm = 0.0028 ppm).

[c] The maximum and mean theoretical residue in feed for lactating goats was estimated to be 4.66 and 1.56 ppm, respectively, assuming the feed is similar to dairy cattle which contained meal, seeds, hulls, and gin trash at 15, 25, 15, and 20%, respectively.

Acute Dietary Exposure

Estimates of potential acute dietary exposure used the highest measured residue values at or below the tolerance for each commodity. The processing and distribution factors described in Tables 18-20 were used to derive the anticipated maximum residue levels for processed cottonseed products, animal tissues, and milk from the maximum residue level for whole cottonseed. The following assumptions were used to estimate potential acute dietary exposure from measured residue values: a) the residue level does not change over time and b) all foods that are consumed will contain the highest residue anticipated.

The acute dietary exposure analyses were conducted using the Exposure-4™ software program developed by Technical Assessment Systems (TAS), Inc. The Exposure-4™ software program estimates the distribution of user-day (consumer-day) exposure for the overall U.S. population and specific population subgroups (TAS, 1996a). A user-day is any day in which at least one food from the specific commodity list is consumed. The consumption analysis uses individual food consumption data from the 1989-91 Continuing Survey of Food Intakes by Individuals (CSFII). Exposure estimates were adjusted for oral absorption (70%).

Based on the 95th percentile of user-days exposures for all specific population subgroups, the potential acute dietary exposures to DEF from all labeled uses (i.e., cotton defoliation) ranged from 249 to 985 ng/kg/day (Table 22). Children, 1 to 6 years old, had the highest potential acute dietary exposure to DEF.

B. EXPOSURE ASSESSMENT (cont.)

Table 22. Estimated Exposure Dosages for Selected Population Subgroups Potentially Exposed to DEF in the Diet Alone or in Combination with Ambient Air[a]

Population Subgroup	ADD[b] (ng/kg)		AADD[c] (ng/kg/day)	
	Diet[d]	Diet+Air[e]	Diet[f]	Diet+Air[g]
U.S. Population - All Seasons	489	599	48	55
Western Region	414	524	42	49
Pacific Region	392	502	41	48
All Infants	309	613	18	38
Nursing Infants (< 1 yr)	555	859	13	34
Non-nursing Infants (< 1 yr)	249	552	19	39
Children (1-6 yrs)	985	1,288	103	123
Children (7-12 yrs)	825	1,128	84	104
Females (13+ yrs/pregnant/not nursing)	384	478	36	43
Females (13+ yrs/nursing)	510	605	43	50
Females (13-19 yrs/not pregnant or nursing)	498	592	47	53
Females (20+ yrs/not pregnant or nursing)	330	424	32	38
Females (13-50 yrs)	390	484	37	43
Males (13-19 yrs)	554	680	61	69
Males (20+ yrs)	403	529	39	47
Seniors (55+ yrs)	287	397	27	35
Workers (M & F, 16+ yrs)	376	486	----	----

[a] Potential dietary sources of DEF include cottonseed oil, meal, and secondary residues in meat and milk.

[b] ADD = Absorbed Daily Dosage assuming 70% oral absorption and 100% respiratory uptake and absorption of DEF as a particulate in ambient air.

[c] AADD = Annual Average Daily Dosage, assuming 70% oral absorption and 100% respiratory uptake and absorption of DEF as a particulate in ambient air.

[d] Based on 95th exposure percentile for all user-day population subgroups.

[e] Based on the upper 95% of ambient air concentration just offsite in the community with the highest detected air concentrations of DEF. The estimated exposure for children (303.5 ng/kg) was used for all infants and children subgroups. The estimated exposure for adult male (125.8 ng/kg) and adult female (94.2 ng/kg) were used for the respective adult male and female subgroups. The exposure dosage for adult males and females (110 ng/kg) was averaged for the U.S. population, western region, Pacific region, seniors, and workers subgroup.

[f] Based on the annual average daily dosage for all population subgroups.

[g] Based on the mean ambient air concentration during use, assuming 60 days of exposure per year. The estimated exposure for children (20.2 ng/kg/day) was used for all infant and children subgroups. The estimated exposure for adult males (8.4 ng/kg/day) and adult females (6.3 ng/kg/day) were used for the respective adult male and female subgroups. The exposure dosages for adult males and females were averaged (7.4 ng/kg/day) for the U.S. population, western region, Pacific region, seniors, and workers subgroup.

B. EXPOSURE ASSESSMENT (cont.)

Chronic Dietary Exposure

The anticipated mean DEF residue levels in processed cottonseed products, animal tissues, and milk are given in Tables 18-20. The following assumptions were used to estimate potential chronic dietary exposure from measured residue values: a) the residue level does not change over time, b) individuals will consume foods that contain the average reported residue, and c) exposures to a commodity at all reported residue levels do occur (i.e., a commodity with the average calculated residue is consumed every day at an annual average level). No adjustment was made for percent crop treated because the dietary residues were theoretical and further refinement did not seem warranted without additional direct measurement of these residues in cottonseed products or their secondary residues in cattle tissue or milk.

The potential chronic dietary exposure was calculated using the Exposure-1™ software developed by TAS (TAS, 1996b). The food consumption data for the chronic analysis were also calculated from the 1989-91 CSFII data. The program estimates the average exposure for all members of a designated population subgroup. Exposure estimates were adjusted for oral absorption (70%).

The mean potential chronic dietary exposure for all population subgroups ranged from 13 to 103 ng/kg/day (Table 22). The population subgroup with the highest potential exposure was children, 1 to 6 years old.

Combined Exposure

The combined exposure to DEF from occupational, dietary and ambient air exposure was also evaluated. The estimated exposure dosages to DEF in ambient air were based on the document prepared to evaluate DEF as a potential toxic air contaminant (Lewis, 1998). The highest estimated exposure in ambient air was just offsite. For workers, the estimated acute, seasonal, and chronic ambient air exposure were 110, 44.5, and 7.4 ng/kg/day, respectively, based on the average offsite exposure for adult males and females in the rural community with the highest air concentrations of DEF. It was assumed DEF was in particulate form in ambient air; therefore, 100% respiratory uptake and absorption was used. The dietary and ambient air contribution to the total exposure for most pesticide workers was minor when compared to their occupational exposure (0.4 to 2.0% for acute exposure, 0.2 to 0.8% for seasonal exposure, and 1.3 to 6.4% for chronic exposure). The dietary and ambient air contribution was greatest among the ground applicators whose occupational exposure was lowest (9.9, 4.2 and 15.5% for acute, seasonal and chronic exposure, respectively). Due to the minor contribution to the total exposure in workers with the highest exposure, no further evaluation of the combined occupational, dietary and ambient air exposure was conducted.

The combined exposure to DEF from diet and ambient air was evaluated for the general population using the estimated exposure for adults and children from the document prepared to evaluate DEF as a potential toxic air contaminant (Lewis, 1998). The highest estimated acute ambient air exposure dosages for children, adult males and adult females were 303.5, 125.8, and 94.2 ng/kg, respectively. The combined acute exposure to DEF from diet and ambient air ranged from 397 ng/kg/day for seniors (male and female) 55 years and older to 1,289 ng/kg/day for children ages 1 to 6 years old. The highest estimated chronic ambient air exposure dosages for children, adult males and adult females were 20.2, 8.4, and 6.3 ng/kg/day, respectively. The

B. EXPOSURE ASSESSMENT (cont.)

combined chronic exposure to DEF from diet and ambient air ranged from 34 ng/kg/day for nursing infants less than one year old to 123 ng/kg/day for children 1 to 6 years old.

C. RISK CHARACTERIZATION

The risk for non-oncogenic health effects in humans is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from experimental animal studies to the human exposure dosage.

$$Margin\ of\ Exposure = \frac{NOEL}{Exposure\ Dosage}$$

Acute Toxicity

The MOEs for dermal irritation were calculated for handlers using the NOEL for dermal irritation for the formulation (8.3 mg formulation/cm^2) and the estimated hand exposure to the formulation in Table 18. The MOEs for dermal irritation ranged from 1,500 for pilots to 37,000 for ground applicators (Table 23).

Since dietary exposure to DEF was minor when compared to the occupational exposure, the MOEs for systemic effects in pesticide workers were calculated only using the ADDs in Table 18 for occupational exposure and the adjusted acute NOEL (4.9 mg/kg). The MOEs ranged from 40 for trampers to 1,114 for ground applicators (Table 23).

For dietary exposure, the MOEs were calculated for the various population subgroups using the adjusted acute NOEL and the acute dietary exposure dosages in Table 22. The MOEs ranged from 5,000 for children, 1 to 6 years old, to 20,000 for non-nursing infants less than 1 year old (Table 24).

For combined dietary and ambient air exposure, the adjusted acute oral NOEL (4.9 mg/kg/day) and combined acute exposure dosages in Table 22 were used to calculate the MOEs. The acute MOEs for combined exposure ranged from 3,800 for children 1 to 6 years old to 12,000 for seniors (male and female) 55 years and older.

Subchronic Toxicity

The MOEs for seasonal occupational exposure for pesticide workers were calculated using the SADD (Table 18) and the adjusted subchronic NOEL (0.95 mg/kg/day). The MOEs ranged from 17 for trampers to 463 for ground applicators (Table 23).

Since exposure to DEF does not appear to vary significantly from season to season, the seasonal dietary exposure in the general population was assumed to be the same as the chronic dietary exposure.

Chronic Toxicity

The MOEs for chronic dietary exposure to DEF were calculated for the various population subgroups using the adjusted chronic NOEL (0.14 mg/kg/day) and the chronic

C. RISK CHARACTERIZATION (cont.)

Table 23. Estimated Margins of Exposure for Pesticide Workers for Potential Acute and Seasonal Exposure to DEF[a]

	Acute		
Workers	Dermal[b]	Systemic[c]	Seasonal[d]
Aerial Application			
Mixer/Loader	4,500	174	72
Pilot	1,500	148	61
Flagger	15,000	206	86
Ground Application			
Mixer/Loader	1,700	90	37
Applicator	37,000	1,114	463
Harvesters			
Picker Operator	------	52	22
Module Builder Operator	------	164	68
Raker	------	88	36
Tramper	------	40	17

[a] Margin of Exposure = NOEL / Exposure Dosage. See Table 18 for exposure dosages for pesticide workers.
[b] The NOEL for dermal irritation was 8.4 mg formulation/cm^2 (rabbits).
[c] The adjusted acute NOEL for systemic effects was 4.9 mg/kg (rats - excessive salivation).
[d] The adjusted subchronic NOEL was 0.95 mg/kg/day (rabbits - muscle fasciculations, brain ChE inhibition, and skin lesions).

dietary exposure dosages in Table 22. The MOEs ranged from 1,400 for children, 1 to 6 years old, to 10,000 for nursing infants less than 1 year old (Table 24).

For combined dietary and ambient air exposure, the adjusted chronic oral NOEL (0.14 mg/kg/day) and combined chronic exposure dosages in Table 22 were used to calculate the MOEs. The chronic MOEs from combined exposure ranged from 1,100 for children 1 to 6 years old to 4,200 for nursing infants less than 1 year old.

Oncogenicity

The risk for oncogenic effects was calculated by multiplying the oncogenic potency by the exposure dosage.

$$Oncogenic\ Risk = Oncogenic\ Potency \times Exposure\ Dosage$$

The oncogenic risk for pesticide workers was calculated using the LADDs in Table 18. The estimated oncogenic potency of DEF based on the incidence of liver hemangiosarcomas in male mice ranged from 3.3×10^{-2} (MLE) to 5.9×10^{-2} (95% UB) $(mg/kg/day)^{-1}$. To correct for

C. RISK CHARACTERIZATION (cont.)

Table 24. Estimated Margins of Exposure for Selected Population Subgroups for Potential Acute and Chronic Exposure to DEF in the Diet Alone or in Combination with Ambient Air[a]

Population Subgroup	Acute[b]		Chronic[c]	
	Diet	Diet + Air	Diet	Diet + Air
U.S. Population	10,000	8,200	3,000	2,500
Western Region	12,000	9,300	3,300	2,834
Pacific Region	13,000	9,800	3,500	2,900
All Infants	16,000	8,000	8,200	3,700
Nursing Infants (<1 yr old)	8,800	5,700	10,000	4,200
Non-Nursing Infants (<1 yr old)	20,000	8,900	7,500	3,600
Children (1-6 yrs)	5,000	3,800	1,400	1,100
Children (7-12)	5,900	4,300	1,700	1,300
Females (13+ yrs/pregnant/not nursing)	13,000	10,000	3,900	3,300
Females (13+ yrs/nursing)	9,600	8,100	3,200	2,800
Females (13-19 yrs/not pregnant/not nursing)	9,800	8,300	3,000	2,600
Females (20+ yrs/not pregnant/not nursing)	15,000	12,000	4,400	3,700
Females (13-50 yrs)	13,000	10,000	3,800	3,200
Males (13-19 yrs)	8,800	7,200	2,300	2,000
Males (20+ yrs)	12,000	9,300	3,600	3,000
Seniors (55+ yrs)	17,000	12,000	5,100	4,000
Workers (M & F, 16+ yrs)	13,000	10,000	------	

[a] Margin of Exposure = Adjusted NOEL / Exposure Dosage. Values are rounded to two significant figures. Potential dietary sources of DEF include cottonseed oil, meal, and secondary residues in meat and milk. See Table 22 for exposure dosages.
[b] The adjusted acute NOEL was 4.9 mg/kg (rats - excessive salivation).
[c] The adjusted chronic NOEL was 0.14 mg/kg (rats - microscopic lesions to the small intestine).

oral absorption, the oncogenic potency was divided by 70%. The resultant adjusted oncogenic potency ranged from 4.7×10^{-2} (MLE) to 8.4×10^{-2} (95% UB) $(mg/kg/day)^{-1}$. The estimated oncogenic risk for pesticide workers ranged 6.8×10^{-6} to 1.9×10^{-4} using the MLE for oncogenic potency (Table 25). When the 95% UB for oncogenic potency was used, the estimated oncogenic risk for workers ranged from 1.2×10^{-5} to 3.4×10^{-4}.

The estimated oncogenic risk from dietary exposure alone was calculated using the chronic exposure for the U.S. population (48 ng/kg/day) and the adjusted oncogenic potency. The estimated oncogenic risk from dietary exposure to DEF ranged from 2.2×10^{-6} (MLE) to 4.0×10^{-6} (95% UB).

C. RISK CHARACTERIZATION (cont.)

Table 25. The Estimated Oncogenic Risk for Pesticide Workers for Potential Lifetime Exposure to DEF[a]

Workers	Maximum Likelihood Estimate	95% Upper Bound
Aerial Application		
Mixer/Loader	4.3×10^{-5}	7.8×10^{-5}
Pilot	5.1×10^{-5}	9.2×10^{-5}
Flagger	3.7×10^{-5}	6.6×10^{-5}
Ground Application		
Mixer/Loader	8.4×10^{-5}	1.5×10^{-4}
Applicator	6.8×10^{-6}	1.2×10^{-5}
Harvesters		
Picker Operator	1.5×10^{-4}	2.6×10^{-4}
Module Builder Operator	4.6×10^{-5}	8.2×10^{-5}
Raker	8.7×10^{-5}	1.6×10^{-4}
Tramper	1.9×10^{-4}	3.4×10^{-4}

[a] Oncogenic Risk = Oncogenic Potency x Exposure Dosage. The exposure dosage was the LADD in Table 18. The maximum likelihood estimate for oncogenic potency after adjusting for oral adsorption was $4.7 \times 10^{-2.}$ The 95% upper bound estimate for oncogenic potency was 8.4×10^{-2}.

The oncogenic risk from combined dietary and ambient air exposure was estimated using the adjusted chronic dietary exposure for the U.S. population (48 ng/kg/day) and the average chronic offsite air exposure for adult males and females (7.4 ng/kg/day). The estimated oncogenic risk from combined exposure to DEF in the diet and ambient air ranged from 2.6×10^{-6} (MLE) to 4.6×10^{-6} (95% UB).

V. RISK APPRAISAL

Introduction

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects observed in toxicity studies with laboratory animals will occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for DEF are delineated in the following discussion.

Hazard Identification

The metabolism of DEF by the various routes of exposure is uncertain since only a few metabolites have been identified. DEF sulfoxide and S,S-dibutyl-S-1-hydroxybutyl phosphorotrithioate were identified in rat urine after intraperitoneal injection of DEF (Hur *et al.*, 1992). A number of metabolites were detected in the urine and feces of several species (rat, goat, chicken) after oral administration of DEF; however, only one metabolite, butyl-gamma-glutamylcysteinylglycine, was identified in rat urine (Kao *et al.*, 1991; Hall, 1991; Sahali, 1991). These investigators suggested that most of the parent compound had been extensively metabolized into natural constituents, such as fatty acids and proteins. nBM was also identified in the excreta of hens administered DEF orally (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators proposed that DEF was hydrolyzed to nBM in the gut causing the late acute effects which were only observed with oral administration of DEF. The hydrolysis of DEF in the gut could be due to either simple degradation or microbial metabolism. Due to the differences in the gastrointestinal tract between birds and mammals, it is unknown if DEF is also easily hydrolyzed to nBM in the gut of mammals. Clinical signs similar to late acute effects in hens have not been observed in mammals; however, similar hematological effects have been observed in a subchronic inhalation study in rats and in chronic feeding studies in mice, rats, and dogs. These hematological changes and the gastrointestinal lesions observed in the chronic feeding studies may be route-specific effects due to nBM rather than DEF. Consequently, these endpoints may not be relevant for occupational exposure in humans which occurs primarily by the dermal route. Although metabolic pathways were proposed based on these few metabolites, the metabolism of DEF by the various routes is still highly speculative.

The physiological role of AChE in the nervous system is well known; however, there is some uncertainty regarding the toxicological significance of brain ChE inhibition because of the poor correlation between the severity of cholinergic signs and the level of ChE inhibition in the brain (U.S. EPA, 1988b). Several factors probably contribute to the poor correlation. One of these factors is that ChE inhibitors produce different degrees of inhibition in the various regions of the brain (Nieminen *et al.*, 1990). Another factor is that some cholinergic signs may be due to peripheral rather than central inhibition of AChE (Murphy, 1986). In addition, brain ChE activity is usually measured at the end of the study whereas the cholinergic signs may be observed at various time points during the study. Often cholinergic signs are observed only at the beginning of the study and then the animals appear to develop a "tolerance" to the ChE inhibitor. This

adaptation or "tolerance" may be due to several possible mechanisms including a “down-regulation” or reduction in the number of post-synaptic receptors (Costa *et al.*, 1982). Finally, clinical observation in animal studies can be a very crude and subjective measurement. Some mild cholinergic signs, such as headaches and anxiety, cannot readily be detected in animals. There may also be other subtle changes in neurological function that will only be detected if the animal is stressed or required to perform certain tasks (Nagymajtényi *et al.*, 1988; Raffaele and Rees, 1990). It is possible that some level of brain ChE inhibition can occur without any untoward effect on neurological function, overt or subtle. However, in the absence of rigorous behavioral and neurophysiological testing, the assumption is made that if there is statistically significant inhibition of brain ChE inhibition, there is probably some deleterious effect to the nervous system. With chronic exposure to DEF, the toxicological significance of brain ChE inhibition was not a major concern because other effects which were clearly adverse (e.g., histological lesions) were seen at the same or lower doses.

A critical NOEL of 7 mg/kg was selected for evaluating the acute occupational, and dietary exposure to DEF was estimated from a developmental toxicity study in rats in which dams receiving DEF at 28 mg/kg/day exhibited excessive salivation on day 3 of dosing (Kowalski *et al.*, 1986). After correcting for oral absorption (70%), the adjusted NOEL was 4.9 mg/kg. This adjusted NOEL was similar to an adjusted acute NOEL that was observed in a 21-day dermal toxicity study based on muscle fasciculations observed in rabbits on day 2 at 29 mg/kg/day (Sheets *et al.*, 1991). After correcting for dermal absorption, the NOEL was reduced from 11 mg/kg to 5.2 mg/kg. Since these adjusted NOELs were so similar, only one was selected to calculate the MOEs for both occupational and dietary exposure. However, both of these NOELs are based on effects that were seen after more than one dose was administered. An acute NOEL could have been estimated from either an acute oral or dermal LD_{50} study by dividing the LOEL by an uncertainty factor of 10. In an oral LD_{50} study, cholinergic signs (decreased activity, lacrimation, salivation, and facial and urogenital stains) were observed in rats after a single oral dose of DEF at 192 mg/kg/day, the lowest dose tested (Sheets, 1991a). In a dermal LD_{50} study, cholinergic signs (ataxia, muscle fasciculations, lacrimation, facial and urogenital stains) were observed in rabbits that received a single application at 500 mg/kg, the lowest dose tested (Sheets and Phillips, 1991). The estimated NOELs (19.2 and 50 mg/kg) were significantly higher than the NOELs for acute effects in the developmental toxicity study or the 21-day dermal toxicity study, suggesting that the second or third dose may have been a significant factor in the development of effects at a lower dose level. However, workers are more likely to be exposed to DEF over several days, rather than on a single isolated day. Therefore, DPR considered it preferable to use the established NOEL for maternal toxicity (excessive salivation) that was observed after 3 days of exposure in the developmental toxicity study versus using an estimated NOEL from an LD_{50} study. However, if an estimated NOEL from one of the LD_{50} studies had been used, the acute MOEs for occupational and dietary exposure would be approximately 2.5 to 5 times higher.

The 21-day dermal toxicity study was also selected as the definitive study for evaluating seasonal occupational exposure to DEF based on muscle fasciculations, brain ChE inhibition (85-86% of controls) and microscopic lesions in the skin at 11 mg/kg/day (Sheets *et al.*, 1991). Several clinical signs (red conjunctiva, lacrimation, and anal stains) and hyperkeratosis were also observed in a few rabbits at the lowest dose level, 2 mg/kg/day. The hyperkeratosis was not considered toxicologically significant because it was of minimal severity and appeared to be reversible after treatment stopped. The other clinical signs were not clearly treatment-related because of their occurrence in control animals. The investigators attributed the red conjunctiva

and lacrimation to the use of plastic collars since these effects were resolved one day after the collars were removed in the recovery groups. Consequently, the lowest dose level was selected as the NOEL rather than the LOEL. If these effects were considered treatment-related, the seasonal MOEs for occupational exposure would be 10-fold lower.

Slightly lower NOELs were observed in two oral studies. In a rat reproductive toxicity study, a NOEL of 0.4 mg/kg/day was observed based on brain ChE inhibition (71% of controls) in adult females (Eigenberg, 1991a). This study was not used for evaluating occupational exposure because of the route of exposure and the uncertainty regarding the impact of the increased DEF intake in the females during lactation on the reduced brain ChE activity at terminal sacrifice. However, if it had been used for evaluating seasonal occupational exposure, the MOEs would be approximately 3-fold lower using the time-weighted average of 0.4 mg/kg/day for the NOEL. Since the DEF intake in females was higher during lactation just prior to their terminal sacrifice, it may be more appropriate to use the average compound consumption during lactation (0.7 mg/kg/day) as the NOEL for females rather than the time-weighted average (0.4 mg/kg/day). If the DEF intake during lactation was used as the critical NOEL, the seasonal MOEs would be only 2-fold lower.

A NOEL of 0.1 mg/kg/day was observed in a 90-day oral neurotoxicity study in hens (Abou-Donia *et al.*, 1979b). This study was not used for a variety of reasons as previously discussed under the Hazard Identification section, the most significant being the uncertainty about the relevance of the mild ataxia observed at the LOEL because of the route of exposure. It is very possible that the mild ataxia is due to nBM (which also causes incoordination) rather than DEF since unequivocal evidence of OPIDN (paralysis and nerve degeneration) were not observed in the hens until 20 mg/kg/day. These same investigators had proposed that DEF is hydrolyzed in the gastrointestinal tract to nBM. If the ataxia is caused by nBM, then this effect is not necessarily relevant to occupational exposure to DEF in humans. However, if this study had been used for seasonal occupational exposure, the MOEs would be approximately 14 times lower than estimated based on 21-day dermal toxicity study in rabbits (Sheets *et al.*, 1991). If this study had been used for evaluating chronic dietary exposure, the MOEs would be approximately 50% lower than estimated based on the 2-year feeding study in rats (Christenson, 1992).

In a 90-week mouse oncogenicity study, brain ChE activity was significantly reduced (91% of control activity) at the lowest dose level, 10 ppm (Hayes, 1989). This reduction was not considered toxicologically significant because no cholinergic signs were seen at 50 ppm and only mild cholinergic signs (loose stools and perianal staining) were observed at 250 ppm. Therefore, the NOEL selected for this study was 10 ppm (M: 1.5 mg/kg/day; F: 2.0 mg/kg/day) based on vacuolar degeneration in the small intestine, hematopoiesis of the spleen, hematological changes, and reduction of ChE activity (87% of controls) at 50 ppm (M: 8.4 mg/kg/day or F: 11.3 mg/kg/day). However, even if the brain ChE inhibition at the 1.5 mg/kg/day had been considered adverse, it would not significantly alter the chronic MOEs because the estimated NOEL, 0.15 mg/kg/day, for this study would be similar to the lowest observed chronic NOEL of 0.2 mg/kg/day from the 2-year rat study (Christenson, 1992).

MOEs were not calculated for chronic occupational exposure because potential long-term health effects (brain ChE inhibition) were either already addressed under subchronic toxicity or they were not considered relevant to occupational exposure to DEF due to differences in duration or route of exposure (hematological changes, lesions in the small intestine, liver and

spleen). Lesions in the eyes and adrenal glands that were observed in rats after subchronic inhalation exposure or chronic oral exposure were not observed in the subchronic dermal study in rabbits. Various factors may have been responsible for the difference in response including a shorter exposure period in the dermal study, lower peak blood levels with dermal exposure and species differences in metabolism or sensitivity. However, even if chronic MOEs had been calculated for occupational exposure using the adjusted chronic NOEL, 0.14 mg/kg/day, they would still be larger than the seasonal MOEs. Although the chronic NOEL was 6.5 times lower than the subchronic NOEL, the chronic exposure was 8 times lower than the seasonal exposure.

There was a significant increase in the incidence of adenocarcinomas in the small intestine of both sexes, in liver hemangiosarcomas in males, and alveolar/bronchiolar adenomas in females in a mouse study; however, there was no evidence of an oncogenic effect in a rat oncogenicity study and the genotoxicity data was negative. Among the tumors seen in mice only the liver hemangiosarcomas in males had an increase in the incidence at doses below the highest dose tested. There was a significant increase in non-neoplastic lesions especially in the small intestine at the high dose suggesting the MTD had been exceeded. At or above the MTD, normal physiology, metabolism and/or repair mechanisms may be overwhelmed, resulting in the initiation or promotion of tumors (Carr and Kolbye, 1991; Swenberg, 1995). Increased cell proliferation due to cytotoxicity can result in the promotion of tumors by decreasing the time available to repair DNA damage. Other nongenotoxic mechanisms, such as immunosuppression or endocrine disruption, could also be responsible for the increase in tumors (MacDonald *et al.*, 1994). If a threshold mechanism, such as increased cell proliferation was involved, the use of a linearized multistage model to estimate oncogenic risk would exaggerate the risk since it assumes there is no biological threshold.

Very little is known about the toxicity of nBM. Only a few studies were available describing the effects in laboratory animals after acute exposure. Some effects observed in animals were indicative of CNS depression including incoordination, muscular weakness, paralysis, lethargy, sedation, respiratory depression, cyanosis, and coma (Fairchild and Stokinger, 1958). Other effects included restlessness, increased respiration, diarrhea, ocular irritation, liver and kidney damage. Evidence of respiratory irritation was seen with inhalation exposure, including sneezing, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage in the lungs. There was insufficient information available in the published report by Fairchild and Stokinger (1958) to establish a NOEL by any of the routes tested.

Abou-Donia and coworkers (1979a & 1984) administered single doses of nBM to hens and observed various clinical including malaise, leg or general weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors, loss of breath, and just prior to death, a dark and droopy comb. The NOEL was 100 mg/kg based on these clinical signs. Abdo *et al*. (1983b) found that hens administered nBM had elevated methemoglobin levels and reduced erythrocyte counts, hematocrit, hemoglobin levels and G-6-PD activity. Because the time course of the hematological changes and the clinical signs were similar, the investigators proposed that the inhibition of G-6-PD was responsible for the hematological changes. A NOEL was not established for the hematological effects in any of the toxicity studies for nBM.

An acute NOEL of 10 ppm (17 mg/kg/day) was established in a developmental toxicity study based on increased mortalities, reduced body weight gain, unkempt appearance, lethargy,

red/brown stains, increased post-implantation losses and fetal malformations in mice. Complaints of nausea, eye and respiratory irritation among residents of communities in cotton-growing regions have been attributed to nBM, which has a strong skunk-like odor (Maddy and Peoples, 1977; Scarborough, 1989). It is not clear if ocular and respiratory irritation were evaluated in the developmental toxicity study in mice. It also does not appear that the mice were evaluated for hematological changes. Therefore, it is possible the acute NOEL for nBM would be lower based on these endpoints.

There were no studies available in which animals were exposed to nBM on a subchronic or chronic basis. Consequently, the potential long-term health effects in humans from seasonal or chronic exposure to nBM are unknown. The long-term health effects from nBM are of particular concern since there is evidence of oncogenicity in mice administered DEF orally. If DEF is significantly hydrolyzed to nBM in the gut of mice as it is in chickens, it is possible that the oncogenicity may be due to the nBM rather than DEF. Additionally, no genotoxicity data were available for nBM either.

Exposure Assessment

Occupational Exposure

With acute exposure, it is preferable to use a high-end estimate such as the 95th percentile. Insufficient information was available in the study selected for harvesters to calculate the 95th percentile. Consequently, the geometric mean or arithmetic mean were used for estimating a single day exposure. Therefore, the estimated MOEs for acute occupational exposure would not cover those people at the upper end of the exposure distribution curve. The exposure for the harvesters may have also been underestimated slightly because it was calculated from predicted cotton boll residues on day 7 and dermal transfer factors estimated from the study conducted by Eberhart and Ellisor (1993) and did not take into consideration inhalation exposure. However, based on the dermal and inhalation exposures on day 15 and 20 of this study, the inhalation exposure represented approximately 5% of the absorbed dose for harvesters.

Several other factors may have resulted in an overestimation of the occupational exposure. The occupational exposure for handlers and harvesters was estimated by extrapolating from a 4-hour monitoring period to a 7-hour or 8-hour work day, respectively. It has been demonstrated that the accumulation of residues on clothing and hands reaches a plateau after the first few hours, so that extrapolating an 8-hour exposure from a 4-hour monitoring period may overestimate exposure by 20-40% (Spencer *et al.*, 1995). Residues on hands, in particular, remained virtually constant over the work day. In addition, the dermal absorption used to estimate the absorbed dosage in workers was based on a dermal absorption study conducted in rats. There is some evidence that the rat can overestimate human dermal absorption by two to ten-fold depending on the chemical (Wester and Maibach, 1977 & 1993; Feldmann and Maibach, 1974; Shah and Guthrie, 1983; Sanborn, 1994; Thongsinthusak, 1993).

Dietary Exposure

The dietary exposure was based entirely on anticipated residues that were estimated from whole cottonseed by using processing and distribution factors derived from limited data on the residues in processed cottonseed products, cattle tissues and milk. The anticipated

residues in cottonseed oil are the least certain since the registrant provided evidence which suggests that most of the DEF residues are removed in a deodorization process. However, no data was provided from samples that had undergone the normal deodorization process; therefore, the anticipated residues in undeodorized oil were used. Elimination of the exposure from cottonseed oil would result in a reduction of the chronic dietary exposure by approximately 75%. Several other assumptions, probably overestimated the chronic dietary exposure for DEF. It was assumed that cattle ate cotton by-products at the maximum level allowed by U.S. EPA (85%) on a long-term basis. Furthermore, only residue levels where DEF was applied at or near the maximum application rate and collected at the shortest allowable preharvest interval were used, although DEF may be applied at a lower rate to cotton and can be harvested from 7 to 21 days post application. Finally, the percent of crop treated was also not factored into the chronic exposure, although there is data indicating that about 65% of the crop is treated (CDFA, 1993; DPR, 1994b &1995; USDA, 1992, 1993, 1994 & 1995). These factors were not included in the calculations because the dietary residues were theoretical and further refinement did not seem warranted without additional direct measurement of these residues in cottonseed products or their secondary residues in cattle tissue or milk.

The 1989-92 CSFII data was used to estimate the dietary consumption of cottonseed byproducts, cattle meat and milk products. The estimate of the 95th percentile exposure in nursing infants less than 1 year old is questionable because the estimate is higher than the estimate for non-nursing infants less than 1 year old who should be consuming more of the products that contain primary or secondary residues of DEF. The chronic exposure estimate (which represents the mean) for the nursing infants appears more reasonable. The chronic exposure estimate for this population subgroup was not only less than the non-nursing infants, it was lower than any other population subgroups. The questionable acute exposure estimate may be a result of the small number of user-days in this population subgroup. There were only 52 user-days out of 153 total person-days (days that participants respond to the survey) in this population subgroup. While this number of user-days appears to be adequate to estimate a mean exposure, it does appear sufficient to estimate exposure at the extremes. The potential acute exposure estimate for non-nursing infants is probably more accurate because of the larger number of person-days (453) and user-days (405).

Combined Exposure

The ambient air exposure dosages were based on air monitoring data from one rural site near Fresno that was less than ½ mile from a cotton field. This site was selected for evaluating ambient air exposure just offsite because it had the highest air concentrations of four sites monitored in the Fresno area. Therefore, these exposure estimates represent a worst case scenario. If the ambient air exposure had been based on air monitoring data from six rural locations in Kern County that were further from the application sites, the exposure dosages would be approximately 80% lower than estimated.

Risk Characterization

Generally, an MOE of at least 100 is considered sufficiently protective of human health when the NOEL for an adverse systemic effect is derived from an animal study. The MOE of 100 allows for humans being 10 times more sensitive than animals and for the most sensitive human being 10 times more sensitive than the average human. The MOEs for acute dermal effects were greater than 1,000 for all pesticide workers. The MOEs for acute systemic effects

were greater than 100 for all workers involved in aerial application, and for ground applicators. However, the acute MOE for mixer/loaders in ground application was less than 100. The acute MOEs were also less than 100 for most cotton harvesters, except module builder operators. The MOEs for seasonal occupational exposure were less than 100 for all pesticide workers, except ground applicators. The acute and chronic MOEs for dietary exposure are all greater than 1,000. The acute and chronic MOEs for combined dietary and ambient air exposure were also all greater than 1,000.

The oncogenic risk estimates for most pesticide workers were between 10^{-4} and 10^{-5}. Estimated dietary oncogenic risk for the U.S. population was between 10^{-5} and 10^{-6}. However, the oncogenic risk has probably been overestimated for chronic dietary exposure because of the conservative assumptions made regarding the residue levels in cottonseed oil, the amount of cotton by-products consumed by cattle and the percent crop treated. The estimated oncogenic risk from combined dietary and ambient air exposure were also between 10^{-5} and 10^{-6}. The oncogenic risk from combined exposure has also been overestimated not only because of conservative assumptions made in the dietary exposure, but also because the ambient air exposure was based on the air monitoring data from one location with the highest air concentrations of DEF.

MOEs were not calculated for nBM because of lack of reliable toxicity data for nBM and no air monitoring data for nBM in workers. The American Conference of Government Industrial Hygienists (ACGIH) threshold limit value (TLV) for nBM is 0.5 ppm (ACGIH, 1986). The TLV is based on a study with ethyl mercaptan in which human volunteers were exposed for 3 hours daily. No complaints were recorded at 1 mg/m^3 (0.4 ppm). A reference exposure level for nBM could also be estimated by dividing the NOEL of 10 ppm (17 mg/kg/day) from the inhalation developmental toxicity study in mice by an uncertainty factor of 100 for interspecies and intraspecies variation in susceptibility. The estimated reference exposure level for nBM is 250 µg/m^3 or 67.8 ppb, assuming a 24-hr respiratory rate of 0.68 m^3/kg/day for a 6-year-old child. The highest daily average air concentration for nBM in ambient air (28.6 µg/m^3 or 7.75 ppb) was reported in the CDFA (1981) study. Therefore, the ambient air concentration is more than 8-fold below the estimated reference exposure level for nBM. However, this ambient air concentration is above the reported odor threshold (0.01 to 1.0 ppb) for nBM (Santodonato *et al.*, 1985). Offensive odors may trigger symptoms in humans, such as nausea and headache, by indirect physiologic mechanisms including exacerbating an underlying medical condition, innate odor aversion, odor-related aversive conditioning, stress-induced illness, and possible innate pheromonal reaction (Shusterman, 1992). Ames and Stratton (1991) found that symptoms more closely correlated with odor perception than distance from a potato field treated with ethoprop which breaks down to n-propyl mercaptan. Theoretically, workers could be exposed to higher air concentrations of nBM during application due to their close proximity to the source. However, it is unclear if the degradation of nBM would be sufficiently rapid that the amounts generated during application would be a health concern for workers.

U.S. EPA's Reregistration Eligibility Document for DEF

U.S. EPA made available in September 1998 a draft of the Health Effects Division (HED) chapter of the Reregistration Eligibility Document (RED) for tribuphos (DEF) on the Internet for public comment (U.S. EPA, 1998) . U.S. EPA evaluated both occupational and dietary exposure to DEF using route-specific NOELs. They evaluated inhalation exposure in workers using the 90-day inhalation study conducted by Pauluhn (1992) with a short-term and

intermediate term NOEL of 0.9 mg/kg/day. DPR did not used this study to evaluate acute or seasonal occupational exposure to DEF since greater than 95% of occupational exposure was dermal even for handlers. U.S. EPA selected the 21-day dermal toxicity study in rabbits for evaluating short-term and intermediate-term exposure to DEF in workers with an estimated NOEL of 0.2 mg/kg/day based on plasma and erythrocyte ChE inhibition (Sheets *et al*, 1991). DPR also selected this study to evaluate seasonal occupational exposure to DEF; however, a different NOEL was identified for this study because of a different science policy regarding the use of blood ChE as a regulatory endpoint. Both agencies do not considered blood ChE inhibition to be an adverse effect in itself; however, U.S. EPA uses it as a surrogate for peripheral ChE inhibition data when peripheral ChE inhibition data is not available. If DPR had used U.S. EPA's dermal NOEL in evaluating the acute occupational exposure, the MOEs would be 25-fold lower than estimated. If this NOEL had been used in evaluating seasonal occupational exposure to DEF, the MOEs would be approximately 5-fold lower than calculated.

U.S. EPA selected a NOEL of 1 mg/kg/day from the developmental toxicity study in rats to evaluate acute dietary exposure to DEF based on plasma and erythrocyte ChE inhibition (Kowalski *et al.*, 1986). DPR used this same study to evaluate acute dietary and occupational exposure to DEF, but identified a higher NOEL of 7 mg/kg/day based on excessive salivation. If DPR has used U.S. EPA's NOEL in evaluating acute dietary and occupational exposure, the MOEs would be 5-fold lower after adjusting for oral absorption (70%). For evaluating chronic dietary exposure, U.S. EPA selected a NOEL of 0.1 mg/kg/day based on plasma ChE inhibition in the 1-year dog study (Christenson, 1991). DPR used a NOEL of 0.2 mg/kg/day based on hyperplasia, and vacuolar degeneration in the small intestine and hematological changes observed in a 2-year rat feeding study (Christenson, 1992). If DPR had used U.S. EPA's NOEL in evaluating chronic dietary exposure to DEF, the MOEs would have been 3-fold lower than estimated.

U.S. EPA had previously used the Abou-Donia *et al.* (1979b) study to calculate an RfD for DEF when many of the acceptable registrant studies were not available. In this draft RED document, the RfD is no longer estimated using the Abou-Donia *et al.* (1979b) study. U.S. EPA identified a NOEL of 11 mg/kg/day for delayed neurotoxicity study in the 90-day neurotoxicity study in hens submitted by the registrant (Sheets, 1991b). This NOEL is higher than the NOEL of 2.6 mg/kg/day that DPR identified for this study based on delayed neuropathy. DPR made the health protective assumption that the slight increase in equivocal lesions at 11 mg/kg/day was treatment-related, even though it was not statistically significant.

As part of the Food Quality Protection Act (FQPA), U.S. EPA evaluated the developmental and reproductive toxicity studies for DEF and concluded, as did DPR, that there was no evidence for increased pre- or post-natal sensitivity. However, they recommended that the 10X uncertainty factor for children be retained because of data gaps. The registrant had not submitted acute and subchronic neurotoxicity studies in rats. U.S. EPA was also requesting a developmental neurotoxicity study in rats based on the delayed neurotoxicity and ocular toxicity caused by DEF and a special 90-day study to further evaluate the ocular toxicity. None of these studies are required under the Birth Defect Prevention Act (SB 950) to register pesticides in California.

U.S. EPA classified DEF as a Likely High Dose/Not Likely Low Dose carcinogen under its new carcinogenicity classification system. Their justification for this classification was that tumors were only increased at the highest dose level where severe toxicity occurred. Although

not explicitly stated, this classification treats DEF is a threshold carcinogen because they use an MOE approach to protect for oncogenicity, rather than calculate an oncogenic potency factor. The NOEL they selected to calculate the MOEs for oncogenicity was 0.1 mg/kg/day based on plasma ChE inhibition in dogs, the most sensitive endpoint for chronic exposure (i.e, the same NOEL used for evaluating the chronic dietary exposure). If DPR had used this approach in evaluating oncogenic risk from occupational exposure to DEF in ambient air, the MOEs for oncogenicity would have ranged from 18 to 700. The MOEs for oncogenicity from dietary exposure to DEF would range from 570 to 2,000.

VI. TOLERANCE ASSESSMENT

A. BACKGROUND

A tolerance is the maximum, legal amount of a pesticide residue that is allowed on a raw or processed agricultural commodity, or in an animal tissue used for human consumption. The U.S. EPA tolerance program was developed as an enforcement mechanism to identify illegal residue concentrations resulting from potential noncompliance with the product label requirements (e.g. improper application rates or methods, inadequate preharvest intervals, direct or indirect application to unapproved commodities). Tolerances are enforced by the FDA, USDA, and state enforcement agencies (e.g., Pesticide Enforcement Branch of DPR).

Current pesticide tolerances are generally set at levels that are not expected to produce deleterious health effects in humans from chronic dietary exposure. The data requirements for establishing a specific tolerance include: 1) toxicology data for the parent compound, major metabolites, degradation products and impurities, 2) product chemistry, 3) analytical method(s) that are readily available, accurate and precise, 4) measured residues in crops used for animals feeds, 5) measured residues in animal tissues (e.g., meat, milk, eggs) from direct or indirect (feed) applications, and 6) measured residue levels from field studies. The minimum requirements for the field study include: 1) an application rate at or above the highest rate on the product label, 2) the greatest number of allowable repeat applications, and 3) the shortest pre-harvest interval listed on the product label. Generally, the registrant of the pesticide requests a commodity-specific tolerance, which is equal to the highest measured residue, or some multiple of that value, from the field trial using the specific pesticide.

Assembly Bill 2161 (Bronzan and Jones, 1989) requires the DPR to "conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides." In the situation where "any pesticide use represents a dietary risk that is deleterious to the health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance . . . " As part of the tolerance assessment, a theoretical dietary exposure for a specific commodity and specific population subgroups can be calculated from the product of the tolerance and the daily consumption rate.

EPA has established tolerances for DEF on cottonseed and cottonseed hulls at 4 and 6 ppm, respectively. Because secondary residues of DEF can occur from feeding cottonseed products to ruminants as roughage, a tolerance level of 0.02 ppm was set for DEF in the meat, meat by-products and fat of cattle, goats and sheep. The tolerance level for milk was set at 0.002 ppm.

B. ACUTE EXPOSURE

An acute exposure assessment using the residue level equal to the tolerance was conducted for each individual label-approved commodity. The TAS Exposure-4 software program and the 1989-91 Continuing Survey of Food Intakes by Individuals (CSFII) were used in this assessment. Tolerances were not established for DEF on cottonseed oil and meal; therefore, the tolerance for whole cottonseed, 4.0 ppm, was used for these two commodities. Initially, the tolerance assessment was conducted considering only the primary residues in cottonseed oil and meal. If the MOEs were larger than 1,000 based on the tolerances for the primary residues, no further analysis of the tolerances for secondary residues was conducted since the amount they would contribute to the total dietary exposure would be negligible compared to the tolerances for primary residues. Using the 95th percentile for acute exposure,

the theoretical maximum residue contribution (TMRC) for DEF ranged from 432 ng/kg/day for seniors, 55 years and older, to 1,486 ng/kg/day for children, 1 to 6 years old (Table 26). The resultant MOEs ranged from 3,300 for children, ages 1 to 6 years old, to 11,000 for seniors, 55 years and older. Since the MOEs were greater than 1,000 for all population groups, no further analysis of secondary residues was warranted. Based on these MOEs, the tolerances for DEF appear to be adequately protective for all population subgroups with regards to acute toxicity.

Table 26. Margins of Exposure from Acute Dietary Exposure to DEF Based on Tolerances

Population Subgroup	Exposure Dosage[a]	Margin of Exposure[b]
U.S. Population	737	6,600
Western Region	638	7,700
Pacific Region	600	8,200
All Infants	993	4,900
Nursing Infants (<1 yr old)	898	5,500
Non-Nursing Infants (<1 yr old)	950	5,200
Children (1-6 yrs)	1,486	3,300
Children (7-12)	1,226	4,000
Females (13+ yrs/pregnant/not nursing)	573	8,600
Females (13+ yrs/nursing)	834	5,900
Females (13-19 yrs/not pregnant/not nursing)	722	6,800
Females (20+ yrs/not pregnant/not nursing)	510	9,600
Females (13-15 yrs)	587	8,400
Males (13-19 yrs)	813	6,000
Males (20+ yrs)	608	8,100
Seniors (55+ yrs)	432	11,000
Workers (M & F, 16+ yrs)	574	8,500

[a] Based on the 95th exposure percentile for all user-day population subgroups, after adjusting for oral absorption (70%).

[b] Margin of Exposure = Adjusted Acute NOEL (4.9 mg/kg) / Dietary Exposure. Values are rounded to two significant figures.

C. CHRONIC EXPOSURE

A chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities has not been conducted because it is highly improbable that an individual would chronically consume single or multiple commodities with

VI. TOLERANCE ASSESSMENT (cont.)

pesticide residues at the tolerance levels. Support for this conclusion comes from the DPR pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residue levels at or above the established tolerance (DPR, 1996b).

Using the tolerance levels for meat, milk, and cottonseed oil, the U.S. EPA (1981) calculated a maximum total daily intake of DEF at 0.1 µg/kg/day for a 16-year-old male weighing 70 kg and 385 µg/kg/day for a 4 kg infant ingesting 770 ml of milk per day. The U.S. EPA stated using the tolerance levels probably overestimated the dietary exposure based on the monitoring data they had available; however, they did not provide any data to support this conclusion.

VII. CONCLUSIONS

The risks for potential adverse human health effects with occupational and dietary exposure to DEF were evaluated. The MOEs for acute dermal effects were greater than 100 for all pesticide workers. The MOEs for acute systemic effects was greater than 100 for all workers involved in application of DEF, except for mixer/loaders in ground application. The acute MOEs for systemic effects were less than 100 for cotton harvesters, except module builder operators. The MOEs for seasonal occupational exposure were less than 100 for all pesticide workers, except ground applicators. The estimated oncogenic risk for pesticide workers was approximately 10^{-4}, except for ground applicators whose estimated oncogenic risk was approximately 10^{-5}. The acute and chronic MOEs for dietary exposure based on anticipated residues in cottonseed products were greater than 1,000 for all population subgroups. The acute and chronic MOEs for combined dietary and ambient air exposure were also greater than 1,000. The estimated oncogenic risk from dietary exposure in the U.S. population was estimated to be between 10^{-5} and 10^{-6}. The estimated oncogenic risk from combined dietary and ambient air exposure were also between 10^{-5} and 10^{-6}. The acute dietary MOEs based on the tolerance for DEF residues on cottonseed were greater than 1,000 for all population subgroups.

VIII. REFERENCES

Abdo, K.M., P.R. Timmons, and M.B. Abou-Donia (Duke Univ. Med. Center, NC), 1983a. Effects of a dermal dose of S,S,S-tri-n-butyl phosphorotrithioate on brain acetylcholinesterase, acid phosphatase, and 2',3'-cyclic nucleotide-3'-phosphohydrolase and plasma butyrylcholinesterase in hens. Dev. Toxicol. Environ. Sci. 11: 499-502. DPR Vol. 272-019, #36680. Also reported in: Abou-Donia, M.B., K.M. Abdo, P.R. Timmons, and J.E. Proctor, 1986. Brain acetylcholines0erase, acid phosphatase, and 2',3'-cyclic nucleotide-3'-phosphohydrolase and plasma butyrylcholinesterase activities in hens treated with a single dermal neurotoxic dose of S,S,S-tri-n-butyl phosphorotrithioate. Toxicol. Appl. Pharmacol. 82(3): 461-473.

Abdo, K.M., P.R. Timmons, D.G. Graham, and M.B. Abou-Donia (Duke Univ. Med. Center, NC), 1983b. Heinz body production and hematological changes in the hen after administration of a single oral dose of n-butyl mercaptan and n-butyl disulfide. Fund. Appl. Toxicol. 3(2): 69-74.

Abou-Donia, M.B. (Duke Univ. Med. Center), 1978. Study of the low level effects of organophosphorous esters with specific reference to the delayed neurotoxic syndrome. Mobay Chem. Corp. Report No. 66057. DPR Vol. 272-016, #28352 & #28353.

Abou-Donia, M.B. (Duke Univ. Med. Center, NC), 1979. Late acute effect of S,S,S-tributyl phosphorotrithioate (DEF) in hens. Toxicol. Letters 4: 231-236. DPR Vol. 272-016, #17247.

Abou-Donia, M.B., 1981. Organophosphorus ester-induced delayed neurotoxicity. Ann. Rev. Pharmacol. Toxicol. 21: 511-548.

Abou-Donia, M.B. and D.M. Lapadula (Duke Univ. Med. Center), 1990. Mechanisms of organophosphorus ester-induced delayed neurotoxicity: type I and type II. Annu. Rev. Pharmacol. Toxicol. 30: 405-440.

Abou-Donia, M.B., D.G. Graham, P.R. Timmons and B.L. Reichert (Duke Univ. Med. Center, NC), 1979a. Delayed neurotoxic and late acute effects of S,S,S-tributyl phosphorotrithioate on the hen: effect of route of administration. Neurotoxicology 1: 425-447. DPR Vol. 272-016, #17245. Also reported in: Abou-Donia, 1978 and Abou-Donia, 1979.

Abou-Donia, M.B., D.G. Graham, K.M. Abdo and A. A. Komeil (Duke Univ. Med. Center, NC), 1979b. Delayed neurotoxic, late acute and cholinergic effects of S,S,S-tributyl phosphorotrithioate (DEF): subchronic (90 days) administration in hens. Toxicol. 14: 229-243. DPR Vol. 272-016, #17243. Also reported in: Abou-Donia, 1978.

Abou-Donia, M.B., D.M. Lapadula, C.D. Carrington and A.A. Nomeir (Duke Univ. Med. Center, NC), 1984. The significance of inhibition of nonspecific esterases in the development of organophosphorus-induced delayed neurotoxicity. In: Cholinesterases - Fundamental and Applied Aspects (Brzin, M., E.A. Barnard and D. Sket, eds), pp 447-461. Walter de Gruyter & Co., Berlin-New York.

VIII. REFERENCES (cont.)

ACGIH, 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, Fifth Edition. American Conference of Governmental Industrial Hygienists Inc., Cincinnati, Ohio.

Ames, R.G. and J.W. Stratton, 1991. Acute health effects from community exposure to n-propyl mercaptan from an ethoprop (Mocap®)-treated potato field in Siskiyou County, California. Arch. Environ. Health. 46(4):213-217.

Astroff, A.B. and S.D. Phillips, 1992a. Acute dermal toxicity study with DEF 6 in rats. Miles Inc. Report No. 102679. DPR Vol. 272-073, #114884.

Astroff, A.B. and S.D. Phillips, 1992b. Experimental acute oral toxicity study with HBM sulfone (a metabolite of tribufos, DEF) in female rats. Miles Inc. Report No. 102684. DPR Vol. 272-082, #116742.

Baron, R.L. and H. Johnson, 1964. Neurological disruption produced in hens by two organophosphate esters. Brit. J. Pharmacol. 23: 295-304. DPR Vol. 272-019, #36678.

Betker, W.R, 1985. The solubility of DEF in various solvents. Mobay Corp. Report No. 90606. DPR Vol. 272-023, #49813.

Brimijoin, S., 1992. Enzymology and biology of cholinesterases. Proceedings of the U.S. EPA Workshop on Cholinesterase Methodologies. Office of Pesticide Programs, U.S. Environmental Protection Agency.

Bronzan and Jones, 1989. Assembly Bill 2161, Addition to the Food and Agriculture Code SEC 8 section 13060. California Food and Agriculture Code, Sacramento, CA.

Carr, C.J. and A.C. Kolbye, Jr., 1991. A critique of the use of the maximum tolerated dose in bioassays to assess cancer risks from chemicals. Regul. Toxicol. Pharmacol. 14: 78-87.

Carrington, C.D., 1989. Prophylaxis and the mechanism for the initiation of organophosphorous compound-induced delayed neurotoxicity. Arch. Toxicol. 63:165-12.

Casida, J.E., R.L. Baron, M. Eto, and J.L. Engel, 1963. Potentiation and neurotoxicity induced by certain organophosphates. Biochem. Pharmacol. 12: 73-83. DPR Vol. 272-019, #36677.

CDFA, 1981. Executive summary of 1980 DEF and butyl mercaptan monitoring study. California Department of Food and Agriculture.

CDFA, 1993. California Field Crops Statistics 1983-1992. California Department of Food and Agriculture, Sacramento, CA. 27pp.

Chemagro Corp., 1965a. DEF residues in cottonseed. Chemagro Corp. Report Nos. 15760, 21225, 21226, 21227, 21240, 21266, 21267 & 24929. CDFA Vol. 272-008, #915062.

Chemagro Corp., 1965b. DEF residues in processed cottonseed. Chemagro Corp. Report Nos. 16379 and 16380. CDFA Vol. 272-008, #915062.

Chemagro Corp., 1968a. DEF residues in cattle tissues. Chemagro Corp. Report Nos. 21889. CDFA Vol. 272-008, #67120.

Chemagro Corp., 1968b. DEF residues in milk. Chemagro Corp. Report Nos. 21892. CDFA Vol. 272-008, #67179.

Chemagro Corp., 1969. DEF residues in cottonseed. Chemagro Corp. Report No. 24780, 24784, 24906, 24907, 24908, 26928 & 24929. CDFA Vol. 272-011, #62995.

Chen, H.H., S.R. Sirianni, and C.C. Huang, 1982a. Sister-chromatid exchanges and cell-cycle delay in Chinese hamster V79 cells treated with 9 organophosphorus compounds (8 pesticides and 1 defoliant). Mutat. Res. 103: 307-313.

Chen, H.H., S.R. Sirianni, and C.C. Huang, 1982b. Sister chromatid exchanges in Chinese hamster cells treated with seventeen organophosphorus compounds in the presence of a metabolic activation system. Environ. Mutag. 4: 621-624.

Christenson, W., 1991. Chronic feeding toxicity study of technical grade tribufos (DEF) with dogs. Mobay Corp. Report No. 100653. CDFA Vol. 272-050, #96421,

Christenson, W., 1992. Technical grade tribufos (DEF): A chronic toxicity/oncogenicity/ neurotoxicity feeding study in the Fischer 344 rat. Miles Inc. Report No. 102675. DPR Vol. 272-072, #114647.

Church, D.D. and H.R. Shaw II, 1969. DEF - Leaching, runoff, and batch adsorption on soils. Mobay Corp. (Chemagro Corp.) Report No. 26292. DPR Vol. 272-012, #62897.

Clemens, G.R., J.J. Bare and R.E. Hartnagel, Jr. (Miles Laboratories, Inc.), 1987. Teratology study in the rabbit with DEF technical. Mobay Corp. Report No. 94468. DPR Vol. 272-027, #58615.

Code of Federal Regulations, 1992. Tolerances and exemptions from tolerances for pesticide chemicals in or on raw agricultural commodities. Title 40, Part 180. Office of the Federal Register National Archives and Records Administration.

Crawford, C.R., 1971. The eye and dermal irritating properties of DEF 6 lbs/gal spray concentrate to rabbits. Chemagro Corp. Report No. 30814. DPR Vol. 272-004, #915122.

Crawford, C.R. and R.H. Anderson, 1972a. The skin and irritation properties of DEF technical to rabbits. Baychem Corp. Report No. 35006. DPR Vol. 272-004, #915121.

Crawford, C.R. and R.H. Anderson, 1972b. The acute oral toxicity of DEF (Special) 6 lbs/gal spray concentrate to rats. Baychem Corp. Report No. 33127. DPR Vol. 272-004, #915110.

Crawford, C.R. and R.H. Anderson, 1972c. The acute dermal toxicity of DEF ULV to rabbits. Baychem Corp. Report No. 34480. DPR Vol. 272-004, #915112.

VIII. REFERENCES (cont.)

Curren, R.D. (Microbiological Associates Inc.), 1989. Unscheduled DNA synthesis in rat primary hepatocytes - DEF technical. Mobay Corp. Report No. 98576. DPR Vol. 272-040, #74444.

Curren, R.D. and P.E. Gentry (Microbiological Associates Inc.), 1989. Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) - DEF technical. Mobay Corp. Report No. 98575. DPR Vol. 272-039, #74445.

Daly, D. (Analytical Bio-chemistry Laboratories, Inc.), 1987. Soil adsorption/desorption with ^{14}C-DEF. Mobay Corp. Report No. 95600. DPR Vol. 272-033, #65385.

De Bleeker, J.L., K.G. Van Den Abeele, J.L. Willems, and J.L. De Reuck, 1992. Are Wistar rats not susceptible to organophosphate-induced delayed neurotoxicity? Res. Commun. Chem. Pathol. 78(2): 253-256.

Dejours P., W.F. Garey, and H. Rahn, 1970. Comparison of ventilatory and circulatory flow rates between animals in various physiological conditions. Resp. Physiol. 9: 108-117.

DPR, 1994a. Case reports received by the California Illness surveillance program in which health effects were attributed to DEF (tribufos) exposure, 1982 to 1991. Pesticide Illness Surveillance Program, Worker Health and Safety Branch, Department of Pesticide Regulation, Sacramento, CA.

DPR, 1994b. Summary of Pesticide Use Report Data, Annual 1992. Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.

DPR, 1995. Summary of Pesticide Use Report Data, Annual 1993. Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.

DPR, 1996a. Summary of Pesticide Use Report Data, Annual 1995. Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.

DPR, 1996b. Residues in Fresh Produce - 1995. Pesticide Enforcement Branch, Department of Pesticide Regulation, California Environmental Protection Agency. 53pp.

D'Harlingue, M.M., 1987. Octanol/water coefficient of DEF. Mobay Corp. Report No. 94682. DPR Vol. 272-028, #55911.

Dubois, K.P. and J. Meskauskas, 1968. The acute inhalation toxicity of a DEF formulation. Univ. of Chicago. DPR Vol. 272-004, #915114.

Eberhart, D.C., 1993. Evaluation of worker exposure to tribufos during aerial and ground applications of DEF 6 to cotton. Miles Inc. DPR Vol. 272-086, #121535.

Eberhart, D.C. and G.K. Ellisor, 1993. Evaluation of worker exposures to tribufos during harvesting of cotton treated with DEF 6. Miles Inc. DPR Vol. 272-087, #121840.

Eigenberg, D.A., 1991a. A two-generation dietary reproduction study in rats using tribufos (DEF). Mobay Corp. Report No. 101255, 1991. DPR Vol. 272-063, #98635.

Eigenberg, D.A., 1991b. A cross-fostering study in rats using tribufos (DEF) administered in the diet. Mobay Corp. Report No. 101254. DPR Vol. 272-062, #98674.

Eigenberg, D.A., 1991c. A dietary reproductive toxicity study investigating the fertility of F_1 rats using tribufos (DEF). Mobay Corp. Report No. 101256. DPR Vol 272-062, #98634.

Ellenhorn, M.J. and D. G. Barceloux, eds., 1988. Pesticides. In: Medical Toxicology: Diagnosis and Treatment of Human Poisoning, pp. 1069-1108. Elsevier, New York. 1512 pp

Fairchild, E.J. and H.E. Stokinger, 1958. Toxicologic studies on organic sulfur compounds. 1. Acute toxicity of some aliphatic and aromatic thiols (mercaptans). Am. Ind. Hyg. Assoc. J. 19: 171-189.

FDA (Division of Pharmacology, Food and Administration, Department of Health, Education and Welfare), 1959. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States, Austin, Texas.

Feldmann, R.J. and H.I. Maibach, 1974. Percutaneous penetration of some pesticides and herbicides in man. Toxicol. Appl. Pharmacol. 28: 126-132.

Fisher, J.R., 1977. Guillain-Barre syndrome following organophosphate poisoning. J. Amer. Med. Assoc. 238(18):1950-1951.

Formoli, T.A. and R.G. Wang, 1995. Estimation of Exposure of Persons in California to Pesticide Products That Contain Tribufos (DEF) (HS-1552). Worker Health and Safety Branch. Department of Pesticide Regulation. California Environmental Protection Agency.

Gaughan, L.C., J.L. Engel, and J.E. Casida, 1980. Pesticide interactions: effects of organophosphorus pesticides on the metabolism, toxicity, and persistence of selected pyrethroid insecticides. Pestic. Biochem. Physiol. 14(1): 81-85.

Grace, T.J. and K.S. Cain (Pharmacology and Toxicology Research Laboratory - West), 1990. Dissipation of tribufos in California soils. Mobay Corp. Report No. 100156. DPR Vol. 272-047, #91795 & 91796.

Hall, L.R., 1991. The metabolism of [^{14}C] and [^{35}S] tribufos in laying hens. Mobay Corp. Report No. 101329. DPR Vol. 272-064, #93263.

Hansen, L.G., B.M. Francis, R.L. Metcalf, J.H. Reinders, and B.L. Bush (Univ. of Illinois), 1982. Effects of chronic oral and dermal administration of pesticides on laying hens, including delayed neuropathy. I. Organophosphorus pesticides. (NCRPIAP Project #145). DPR Vol. 272-016, #17244. Also reported in: Francis, B.M., R.L. Metcalf, and L.G. Hansen, 1985. Toxicity of organophosphorus esters to laying hens after oral and dermal administration. J. Environ. Sci. Health [B] 20(1): 73-95.

Harris, L.E. (Harris Laboratories, Inc.), 1965. Report on demyelination studies on hens - DEF (S,S,S-tributyl phosphorotrithioate). Mobay Corp. Report No. 16614. DPR Vol. 272-005, #915145.

VIII. REFERENCES (cont.)

Hayes, R.H., 1985. Pilot study on technical tribufos (DEF) with mice - Study Number 85-971-01. Mobay Corp. Report 90445. DPR Vol. 272-020, #38470.

Hayes, R.H., 1989. Oncogenicity study of technical grade DEF (tribufos) with mice. Mobay Corp. Report No. 99175. DPR Vol. 272-041, #76144.

Hur, J.H., S.-Y. Wu, and J.E. Casida, 1992. Oxidative chemistry and toxicology of S,S,S-tributyl phosphorotrithioate (DEF defoliant). J. Agric. Food Chem. 40: 1703-1709.

Inui, K., K. Mitsumori, T. Harada, and K. Maita, 1993. Quantitative analysis of neuronal damage induced by tri-*ortho*-cresyl phosphate in Wistar rats. Fund. Appl. Toxicol. 20(1): 111-119.

Jackson, S.B., A. Kesterson, and L.L. Lawrence (Pharmacology and Toxicology Research Laboratory), 1988. Soil surface photolysis of [^{14}C]DEF in natural sunlight. Mobay Corp. Report No. 95673. DPR Vol. 272-034, #95673.

Johnson, M.K., 1970a. Examination of DEF (technical grade tributylphosphorotrithioate) from Chemagro Corporation for delayed neurotoxicity. Mobay Corp. Report No. 27690. DPR Vol. 272-005, #915127.

Johnson, M.K., 1970b. Organophosphorus and other inhibitors of brain 'neurotoxic esterase' and the development of delayed neurotoxicity in hens. Biochem. J. 120: 523-531. DPR Vol. 272-019, #36679.

Kalow, W. and R.O. Davies, 1958. The activity of various esterase inhibitors towards atypical human serum cholinesterase. Biochem. Pharmacol. 1: 183-192.

Kao, L-R.M., R.N. Midden, L.L. Bosnak, and M.E. Krolski, 1991. Disposition and metabolism of [1-^{14}C] tribufos in rats. Mobay Corp. Report No. 101331. DPR Vol 272-064, #93265.

Kenley, R.A., R.A. Howd, and E.T. Uyeno, 1982. Effects of PAM, proPAM, and DFP on behavior, thermoregulation, and brain AChE in rats. Pharmacol. Biochem. Behav. 17: 1001-1008.

Kesterson, A. and L.L. Lawrence (Pharmacology and Toxicology Research Laboratory), 1990. Solution photolysis of [^{14}C]DEF in natural sunlight. Mobay Corp. Report No. 99730. DPR Vol. 272-042, #86140.

Kilgore, W., C. Fischer, J. Rivers, N. Akesson, J. Wicks, W. Winters, and W. Winterlin, 1984. Human exposure to DEF/merphos. Residue Rev. 91: 71-101.

Kimmerle, G., 1972. DEF ULV 6 lb./gal. S.C. - acute inhalation toxicity. Bayer AG Report No. 34161. DPR Vol. 272-004, #915115.

Kowalski, R.L., G.R. Clemens, J.J. Bare and R.E. Hartnagel Jr. (Miles Laboratories, Inc.), 1986. A teratology study with DEF technical in the rat. Mobay Corp. Report No. 87320. DPR Vol. 272-024, #50639.

Lapadula, D.M., C.D. Carrington and M.B. Abou-Donia, 1984. Induction of hepatic microsomal cytochrome P-450 and inhibition of brain, liver, and plasma esterases by an acute dose of S,S,S-tri-n-butyl phosphorotrithioate (DEF) in the adult hen. Toxicol. Appl. Pharmacol. 73: 300-310. DPR Vol. 272-014, #62115.

Lefkowitz, R. J., B. B. Hoffman and P. Taylor, 1990. Neurohumoral Transmission: The Autonomic and Somatic Motor Nervous Systems. In: Goodman and Gilman's: The Pharmacological Basis of Therapeutics 8th Edition (Gilman, A.G., T. W. Rall, A. S. Nies and P. Taylor, Eds.), pp. 84-130. Pergamon Press, New York. 1811 pp.

Leimkuehler, W.M., 1980 (Revised in 1987). DEF water solubility at 20°C and 30°C. Mobay Corp. Report No. 68926. DPR Vol. 272-028, #55910.

Levi, P.E. and E. Hodgson, 1985. Oxidation of pesticides by purified cytochrome P-450 isozymes from mouse liver. Toxicol. Letters (AMST). 24(2-3): 221-228.

Lewis, C.M., 1998. S,S,S-Tributyl Phosphorotrithioate (DEF) As a Toxic Air Contaminant, Part C, Health Assessment. Medical Toxicology Branch, Department of Pesticide Regulation, California Environmental Protection Agency. November 3, 1998.

Lockridge, O. 1990. Genetic variants of human serum cholinesterase influence metabolism of the muscle relaxant succinylcholine. Pharmacol. Ther. 47(1): 35-60.

Lotti, M., 1992. The pathogenesis of organophosphate polyneuropathy. Crit. Rev. Toxicol. 21(6): 465-487.

Lotti, M., C.E. Becker, M.J. Aminoff, J.E. Woodrow, J.N. Seiber, R.E. Talcott, and R.J. Richardson, 1983. Occupational exposure to the cotton defoliants DEF and merphos - a rational approach to monitoring organophosphorous-induced delayed neurotoxicity. J. Occup. Med. 25(7): 517-522.

MacDonald, J.S., G.R. Lankas, and R.E. Morrissey, 1994. Toxicokinetic and mechanistic considerations in the interpretation of the rodent bioassay. Toxicol. Pathol. 22(2): 124-140.

Maddy, K.T. and S.A. Peoples, 1977. Summary of Observations Made in California Concerning Illnesses of Persons Living, Working or Going to School on Property Near a Cotton Field Recently Sprayed with a Defoliant. California Department of Food and Agriculture, Worker Health and Safety Branch, Sacramento, CA. HS-418.

McLeod, W.R., 1975. Merphos poisoning or mass panic? Aust. New Zea. J. Psychia. 9: 225-229.

Mirakhmedov, A.K., K.R. Ochilov, G.A. Sagatova, M.Z. Khan and V. Khole, 1989. Effect of defoliant (butiphos) on morpho-physiological properties and enzyme systems of natural membranes. Ind. J. Exp. Biol. 27: 245-247.

Moretto, A., E. Capodicasa, and M. Lotti, 1992. Clinical expression of organophosphate-induced delayed polyneuropathy in rats. Toxicol. Letters 62: 97-102.

Murphy, S.D., 1986. Toxic effects of pesticides. In: Casarett and Doull's Toxicology - The Basic Science of Poisons, 3rd ed. (Klaassen, C.D., M.O. Amdur and J. Doull, eds.). Macmillan Publishing Co., New York. pp. 519-581.

Murphy, S.D., K.L. Cheever, S.Y.K. Chow and M. Brewster, 1976. Organophosphate insecticide potentiation by carboxylesterase inhibitors. Proc. Eur. Soc. Toxicol. Predict. Chronic Toxicol. Short Term Stud., Prod. Meet., 1975. 17: 292-300.

National Agricultural Chemicals Association, 1990. MSDS Reference for Crop Protection Chemicals, 3rd ed. Chemical and Pharmaceutical Press. pp 768-770, 1063-1065.

Nicholas, A.H. and H. Van Den Berghe, 1982. Sister chromatid exchange and pesticides, with emphasis on organophosphates. In: Sister Chromatid Exchange. Prog. Top Cytogenet. 2: 327-354.

Obrist, J.J. and J.S. Thornton, 1978. Conversion of Folex to DEF on cotton leaves. Mobay Corp. Report No. 66114. DPR Vol. 272-015, #62145.

Olson, G.L., J.D. Marsh, and L.J. Lawrence (Pharmacology and Toxicology Research Laboratory), 1989. Anaerobic metabolism of [^{14}C]DEF in a sandy loam soil. Mobay Corp. Report No. 99729. DPR Vol. 272-042, #86139.

Olson, G.L., J.D. Marsh, and L.J. Lawrence (Pharmacology and Toxicology Research Laboratory), 1990. Aerobic metabolism of [^{14}C]DEF in sandy loam soil. Mobay Corp. Report No. 99728. DPR Vol. 272-042, #86138.

Padilla, S. and B. Veronesi, 1988. Biochemical and morphological validation of a rodent model of organophosphorus-induced delayed neuropathy. Toxicol. Ind. Health. 4(3): 361-371.

Padilla, S., V.C. Moser, C.N. Pope and W.S. Brimijoin, 1992. Paraoxon toxicity is not potentiated by prior reduction in blood acetylcholinesterase. Toxicol. Appl. Pharmacol. 117: 110-115.

Pantuck, E.J., 1993. Plasma cholinesterase: gene and variations. Anesth. Analg. 77(2): 380-386.

Pauluhn, J. (Bayer AG), 1991. DEF (Common names: tribufos; tribufate, suggested) Range-finding study of the subacute inhalation toxicity to rats (Exposure: 10 x 6h). Mobay Corp. Report No. 100695. DPR Vol. 272-056, #97998.

Pauluhn, J. (Bayer AG), 1992. DEF (Common name: tribufos) Study of the subchronic inhalation toxicity to rats in accordance with OECD guideline No. 413. Mobay Corp. Report No. 102697. DPR Vol. 272-081, #116231.

Peoples, S.A., K.T. Maddy, P.R. Datta, L. Johnston, C. Smith, and C. Cooper, 1981. Monitoring of Potential Exposures of Mixer-Loaders, Pilots, and Flaggers During Application of Tributyl Phosphorotrithioate (DEF) and tributyl phosphorotrithioite (Folex)

to cotton fields in the San Joaquin Valley in California in 1979. California Department of Food and Agriculture, Worker Health and Safety Branch, Sacramento, CA.

Peto R., M.C. Pike, N.E. Day, R.G. Gray, P.N. Lee, S. Parish, J. Peto, S. Richards, and J. Wahrendorf, 1980. Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: Long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs on Evaluation of the Carcinogenic Risk of Chemicals to Humans. Supplement 2. IARC, Lyon, France, pp 340-345.

Putman, D.L. and M.J. Morris (Microbiological Associates Inc.), 1989. Chromosome aberrations in Chinese hamster ovary (CHO) cells - DEF technical. Mobay Corp. Report No. 98592. DPR Vol. 272-039, #74161.

Ray, D.E., 1980. Selective inhibition of thermogenesis by tributyl S,S,S-phosphorotrithioate (DEF). Br. J. Pharmacol. 69(2): 257-264.

Ray, D.E. and V.J. Cunningham, 1985. Hypothermia produced by tributyl S,S,S-phosphorotrithioate (DEF). Arch. Toxicol. 56(4): 279-282.

Root, M.S. and J. Doull, 1966. Comparative subacute oral toxicity of some organic phosphates in rats and dogs. Presented at the Fifth Annual Meeting of the Society of Toxicology. Mobay Corp. Report No. 17845. DPR Vol. 272-004, #915108.

Root, M., J. Meskauskas, and J. Doull, 1967. Chronic oral toxicity of DEF to male and female rats. Mobay Corp. Report No. 20462. DPR Vol. 272-005, #915138.

Sahali, Y., 1991. Metabolism of [1-^{14}C] tribufos in lactating goats. Mobay Corp. Report No. 101330. DPR Vol. 272-064, #93264.

Sanborn, J.R., 1994. Human exposure assessment for propoxur. Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency.

Santodonato, J., S. Bosch, W. Meylan, J. Becker, and M. Neal, 1985. Monograph on human exposure to chemicals in the workplace: mercaptans. Syracuse Research Corp., Syracuse, NY. Prepared for National Cancer Institute. NTIS No. PB86-155090.

Satoh, T. and K.P. DuBois, 1973. A liver arylamidase extremely sensitive to organophosphorus compounds. Proc. Symp. Drug Metab. Action. 5: 163-173.

Scarborough, M.E., R.G. Ames, M. Lipsett, and R.J. Jackson, 1989. Acute Health Effects of Community Exposure to Cotton Defoliants. California Department of Health Services, Berkeley, CA.

Schocken, M.J. and G.D. Parker, 1987. Leaching of aged soil residues of DEF in California sandy loam. Mobay Corp. Report No. 95016. DPR Vol. 272-046, #91797.

Schocken, M.J. and I. Philippson, 1987. Stability of DEF in sterile aqueous buffer solutions. Mobay Corp. Report No. 94918. DPR Vol. 272-032, #64718.

Schroeder, S.R., 1992. Dermal absorption of tribufos by rats from a DEF 6 emulsifiable formulation using ^{14}C-tribufos. Miles Inc. Report No. 102681. DPR Vol. 272-074, #114925.

Shah, P.V. and F.E. Guthrie, 1983. Percutaneous penetration of three insecticides in rats: a comparison of two methods for *in vivo* determination. J. Invest. Dermatol. 80: 292-293.

Sheets, L.P., 1990. Dermal sensitization study with technical grade DEF (tribufos) in guinea pigs. Mobay Corp. Report No. 100268. DPR Vol. 272-045, #91422.

Sheets, L.P., 1991a. Acute oral toxicity study of technical grade tribufos (DEF) in rats. Mobay Corp. Report No. 100697. DPR Vol. 272-056, #97996.

Sheets, L.P., 1991b. Subchronic delayed neurotoxicity study with technical grade tribufos (DEF) in hens. Mobay Corp. Report No. 100006. CDFA Vol. 272-051, #89360.

Sheets, L.P. and M.K. Fuss, 1991. Primary dermal irritation study with technical grade tribufos (DEF) in rabbits. Mobay Corp. Report. No. 100694. DPR Vol. 272-054, #97415.

Sheets, L.P. and S.D. Phillips, 1991. Acute dermal toxicity study with technical grade tribufos (DEF) in rabbits. Mobay Corp. Report No. 100698. DPR Vol. 272-056, #97997.

Sheets, L.P., S.D. Phillips and S.G. Lake, 1991. 21-Day dermal toxicity study with technical grade tribufos (DEF) in rabbits. Mobay Corp. Report No. 101279. DPR Vol. 272-061, #98276.

Sheets, L.P. and S.D. Phillips, 1992a. Primary eye irritation study with technical grade tribufos in rabbits. Miles Inc. Report No. 102674. DPR Vol 272-073, #114924.

Sheets, L.P. and S.D. Phillips, 1992b. Acute oral toxicity study with DEF 6 in rats. Miles Inc. Report No. 102678. DPR Vol. 272-073, #114876.

Sheets, L.P. and S.D. Phillips, 1992c. Primary dermal irritation study with DEF 6 in rabbits. Miles Inc. Report No. 102663. DPR Vol. 272-070, #113994.

Shusterman, D., 1992. Critical review: the health significance of environmental odor pollution. Arch. Environ. Health 47(1):76-87.

Somkuti, S.G., H.A. Tilson, H.R. Brown, G.A. Campbell, D.M. Lapadula, and M.B. Abou-Donia, 1988. Lack of delayed neurotoxic effect after tri-*o*-cresyl phosphate treatment in male Fischer 344 rats: biochemical, neurobehavioral, and neuropathological studies. Fund. Appl. Toxicol. 10: 199-205.

Spencer, J.R., J.R. Sanborn, B.Z. Hernandez, R.I. Krieger, S.S. Margetich, and F.A. Schneider, 1995. Long vs. short monitoring intervals for peach harvesters exposed to foliar azinphos-methyl residues. Toxicol. Lett. 78: 17-24.

Swenberg, J.A., 1995. Bioassay design and MTD setting: old methods and new approaches. Regul. Toxicol. Pharmacol. 21: 44-51.

Talbott, T.D., 1987. Henry constant of DEF pure active ingredient. Mobay Corp. Report No. 91256. DPR Vol. 272-029, #60244.

Talbott, T.D., 1990. Product chemistry of DEF technical. Mobay Corp. Reports Nos. 90606, 94500, 94682, 94689, 94806. DPR Vol. 272-044, #91415.

Talbott, T.D. and B. Mosier, 1987. Vapor pressure of DEF pure active ingredient. Mobay Corp. Report No. 94689. DPR Vol. 272-029, #60243.

TAS, 1996a. Exposure 4™, Detailed Distributional Dietary Exposure Analysis. Version 3.35 (1989-92 CSFII). Technical Assessment Systems, Inc., Washington, D.C.

TAS, 1996b. Exposure 1™, Chronic Dietary Exposure Analysis. Version 3.35 (1989-92 CSFII). Technical Assessment Systems, Washington, D.C.

Thomas, W.C., J.A. Secker, J.T. Johnson, C.E. Ulrich, D.R. Klonne, J.L. Schardein and C.J. Kirwin, 1987. Inhalation teratology studies of n-butyl mercaptan in rats and mice. Fund. Appl. Toxicol. 8: 170-178.

Thongsinthusak, T., 1994. Guthion, dermal absorption study. Review Memorandum. Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency.

Thornton, J.S., 1968. Effect of the oil deodorization process on DEF residues in cottonseed oil (simulated). Chemagro Corp. Report No. 21888. CDFA 272-008, #91592.

Thyssen, J. 1976. DEF - Neurotoxicity studies on hens. Bayer AG Report No. 5948. DPR Vol. 272-005, #915134.

Thyssen, J., 1978a. DEF - Studies on acute inhalation toxicity (rats). Bayer AG Report No. 7636. DPR Vol. 272-005, #915116.

Thyssen, J., 1978b. DEF - Subacute inhalation study on rats. Bayer AG Report 7649. DPR Vol. 272-001, #915133.

Thyssen, J. and B. Schilde, 1976a. DEF - Neurotoxicity studies on hens (inhalation experiments). Bayer AG. Report No. 6444. DPR Vol. 272-005, #915136.

Thyssen, J. and B. Schilde, 1976b. DEF - Neurotoxicity studies on dermally treated hens. Bayer AG Report No. 6255. DPR Vol. 272-005, #915135.

Thyssen, J. and B. Schilde, 1978a. DEF - Neurotoxic effects on poultry (subacute inhalation study). Bayer AG Report No. 7614. DPR Vol. 272-005, #915129.

Thyssen, J. and B. Schilde, 1978b. DEF - Studies of subacute dermal neurotoxic effects on poultry. Bayer AG Report No. 8031. DPR Vol. 272-005, 915137.

Thyssen, J., W. Wechsler, and B. Schilde, 1977. DEF - Neurotoxicity studies in hens (thirty-day feeding experiments). Bayer AG Report No. 6941. DPR Vol. 272-005, #915128. Second experiment also reported in: Thyssen, J. and B. Schilde, 1976. DEF -

Neurotoxicity studies on hens (30-day feeding study). Bayer AG Report No. 6440. DPR Vol. 272-025, #57534.

USDA, 1989-1991. Food and Nutrient Intake of Individuals in the United States, 1 Day, 1989-1992. Continuing Survey of Food Intakes by Individuals, 1989-1992. U.S. Department of Agriculture, Agricultural Research Service.

USDA, 1992. Agricultural Chemical Usage 1991 Field Crops Summary. National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C. 150 pp.

USDA, 1993. Agricultural Chemical Usage 1992 Field Crops Summary. National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C. 118 pp.

USDA, 1994. Agricultural Chemical Usage 1993 Field Crops Summary. National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C. 114 pp.

USDA, 1995. Agricultural Chemical Usage 1994 Field Crops Summary. National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C. 106 pp.

U.S. EPA, 1981. Decision Document - DEF. Office of Pesticide Programs, U.S. E.P.A, Washington, D.C. 42 pp.

U.S. EPA, 1993. An SAB Report: Cholinesterase Inhibition and Risk Assessment. Review of the Risk Assessment Forum's Draft *Guidance on the Use of Data on Cholinesterase Inhibition in Risk Assessment by the SAB/SAP Joint Committee.* U.S. Environmental Protection Agency. EPA-SAB-EHC-93-011.

U.S. EPA, 1994. Pesticide Reregistration Rejection Rate Analysis Residue Chemistry - Follow-up Guidance for: Updated Livestock Feeds Tables, Aspirated Grain Fractions (Grain Dust): A Tolerance Perspective, Calculating Livestock Dietary Exposure, and Number and Location of Domestic Crop Field Trials. Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 1995. Office of Pesticide Programs Reference Dose Tracking Report (9/10/95). Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 1998. Memorandum: The HED Chapter of the Reregistration Eligibility Document (RED) for Tribuphos. PC Code 074801; List B, Case No. 2145, DP Barcode 222993. Office of Prevention, Pesticides and Toxic Substances. U.S. Environmental Protection Agency. (Website: www.epa.gov/pesticides/op/group2/tribufos/trihed.pdf)

Veronesi, B., S. Padilla, K. Blackman, and C. Pope, 1991. Murine susceptibility to organophosphorus-induced delayed neuropathy (OPIDN). Toxicol. Appl. Pharmacol. 107: 311-324.

Ware, G.W., 1978. The Pesticide Book. W.H. Freeman and Co., San Francisco. p. 92.

Warren, D.L., 1990. Acute four-hour inhalation toxicity study with technical grade DEF in rats. Mobay Corp. Report No. 100593. DPR Vol. 272-049, #96199.

Warren, D.L. and A.T. Tran, 1992. Acute four-hour inhalation toxicity study with DEF 6 in rats. Miles Inc. Report No. 102669. DPR Vol. 272-071, #114171.

Wester, R.C. and H.I. Maibach, 1977. Percutaneous absorption in man and animal. In: *Cutaneous Toxicity*, Drill V. and P. Lazer (eds). Academic Press, New York.

Wester, R.C. and H.I. Maibach, 1993. Animal models for percutaneous absorption. In: *Risk Assessment: Dermal and Inhalation Exposure an Absorption of Toxicants*, Wang, R.G.M., J.B. Knaak, and H.I. Maibach (eds). CRC Press, Boca Raton.

White, P.D., D.E. Carter, D. Earnest and J. Mueller, 1980. Absorption and metabolism of three phthalate diesters by the rat small intestine. Food Cosmet. Toxicol. 18(4): 383-386.

Wilson, B.W., C.M. Cisson, W.R. Randall, J.E. Woodrow, J.N. Seiber, and J.B. Knaak, 1980. Organophosphate risk assessment: field testing of DEF with the scaleless chicken. Bull. Environm. Contam. Toxicol. 24: 921-928. DPR Vol. 272-016, #17246. Also reported in: Wilson, B.W., C.M. Cisson, W.R. Randall, J.E. Woodrow, J.N. Seiber and J.B. Knaak, 1982. An animal model for testing organophosphates in the field - S,S,S-tributyl phosphorotrithioate and the scaleless chicken. ACS Symp. Ser. 182 (Pestic. Residues Exposure): 189-200.

Wing, K.D., A.H. Glickman, and J.E. Casida, 1984. Phosphorothiolate pesticides and related compounds: oxidative bioactivation and aging of the inhibited acetylcholinesterase. Pesticide and Biochem. Physiol. 21(1): 22-30.

Zielhuis, R.L. and F.W. van der Kreek, 1979. The use of a safety factor in setting health based permissible levels for occupational exposure. Int. Arch. Occup. Environ. Health. 42: 191-201.

APPENDICES

APPENDIX A Equations for Inhalation Studies

APPENDIX B Oncogenicity Computer Model Printout

APPENDIX A - EQUATIONS FOR INHALATION STUDIES

1. Dose estimation for animals from an inhalation study when exposure level is in mg/m^3:

$$dose\ (mg/kg/day) = mg/m^3 \times RR_a \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} \times AF$$

2. Dose estimation for animals from an inhalation study when exposure level is in ppm:

$$dose\ (mg/kg/day) = ppm \times \frac{M.Wt.}{M.Vol.} \times RR_a \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} \times AF$$

NOTE: 1 mg/m^3 = 1 µg/liter
1 ppm = 1 µg/ml
M.Wt. = molecular weight in grams
M.Vol. = molecular volume which is 24.45 liters at 25°C
RR = respiratory rate in m^3/kg/day where a is for animal and h is for human.
AF = respiratory retention/absorption factor

APPENDIX B

Oncogenicity Computer Model Printout

DATE: 04-05-96 TIME: 09:09:17

MULTI-WEIB (MAR 1985)

K.S. CRUMP & COMPANY, INC.
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

Liver Hemangiosarcomas in Males - Term. Sac. Nonfatal

THE 16 OBSERVATIONS AT LEVEL 1 WITH A DOSE OF .000000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
25.0	1	0	51.0	1	0
53.0	1	0	61.0	1	0
72.0	1	0	76.0	1	0
78.0	1	0	80.0	1	0
84.0	1	0	84.0	1	3
85.0	1	0	87.0	2	0
88.0	1	0	89.0	1	0
90.0	1	0	91.0	34	0

THE 14 OBSERVATIONS AT LEVEL 2 WITH A DOSE OF .220000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
49.0	1	0	51.0	1	0
55.0	1	0	57.0	1	0
60.0	1	0	65.0	1	0
72.0	1	0	79.0	2	0
81.0	1	0	82.0	1	0
83.0	1	0	90.0	2	0
91.0	35	0	91.0	1	2

THE 19 OBSERVATIONS AT LEVEL 3 WITH A DOSE OF 1.21000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
44.0	1	0	47.0	1	0
53.0	1	0	59.0	1	3
64.0	1	0	64.0	1	3
68.0	1	0	69.0	1	0
70.0	1	0	75.0	1	0
80.0	1	0	81.0	1	3
81.0	1	0	83.0	1	0
87.0	3	0	88.0	3	0
89.0	1	0	91.0	28	0
91.0	1	2			

THE 26 OBSERVATIONS AT LEVEL 4 WITH A DOSE OF 6.91000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
25.0	1	0	32.0	1	0
47.0	1	0	52.0	1	0
64.0	1	0	69.0	2	0
70.0	1	0	72.0	1	0
73.0	1	0	74.0	1	0
75.0	2	3	75.0	3	0
76.0	1	0	77.0	1	0
77.0	1	3	78.0	1	0
79.0	2	3	83.0	1	0
85.0	1	0	85.0	1	3
87.0	2	0	88.0	1	0
89.0	1	0	90.0	1	0
91.0	19	0	91.0	1	2

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp((-Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3) * (T - T0)^J)

THE MAXIMUM LIKELIHOOD ESTIMATION OF:

PROBABILITY FUNCTION COEFFICIENTS

Q(0)= .143009876262E-09
Q(1)= .169936984452E-09
Q(2)= .000000000000
Q(3)= .000000000000

TIME FUNCTION COEFFICIENTS

T0 = 24.9999090000
J = 4.55852108337

THE MAXIMUM LIKELIHOOD IS -31.4923915518

MAXIMUM LIKELIHOOD ESTIMATES OF EXTRA RISK

****

WEIBULL LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

****

RISK	MLE DOSE	LOWER BOUND ON DOSE	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME
1.000000E-06	2.987309E-05	1.666645E-05	1.792408E-06	95.0%	91.0000

WEIBULL UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

CONFIDENCE

DOSE	MLE RISK	UPPER BOUND ON RISK	LIMIT INTERVAL	TIME
1.00000	3.292087E-02	5.901792E-02	95.0%	91.0000

NORMAL COMPLETION!